THE EFFECTS OF DIETARY ESSENTIAL FATTY ACID ENRICHMENT ON THE NUTRITION AND CONDITION OF RED KING CRAB (*PARALITHODES CAMTSCHATICUS*) LARVAE

By

Asia Beder

RECOMMENDED:

Dr. Louise Copeman

Dr. Sherry Tamone

Dr. Ginny Eckert
Advisory Committee Chair

Dr. Franz Mueter
Chair, Graduate Program in Fisheries

APPROVED:

Dr. Joan Braddock
Interim Dean, School of Fisheries and Ocean Sciences

Dr. John Eichelberger
Dean of the Graduate School

7 December 2015
Date

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Asia Beder, B.S.

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Abstract

Alaska red king crab (*Paralithodes camtschaticus)* is a commercially valuable crab species in Alaska; however, six of the nine red king crab stocks in Alaska are depleted and have failed to recover even after closures of the fisheries. Hatchery rearing is being explored as a possibility for rehabilitating these stocks. In Chapter 1 I review the importance of diet, particularly fatty acids (FAs), when raising crustacean larvae in hatcheries. Long-chain polyunsaturated FAs (PUFAs) are important in molting success, growth, ability to handle stress, and overall survival of crustaceans. Specifically, when the FAs docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are added to the diet, crustacean survival increases. In Chapter 2 I performed a live-food enrichment experiment in which I tested the importance of dietary ratios of DHA, EPA and AA to red king crab larval growth, survival, and energy storage. *Artemia* nauplii that were fed to king crab larvae were enriched with four experimental diets, including 1) DHA, 2) DHA & EPA, 3) DHA & AA, and 4) a control diet that had the same total lipid per wet weight but was enriched with oleic acid (OA, 18:1n-9) and had very low PUFAs. Larval condition, assessed by viewing size and number of lipid droplets, was significantly greater at the glaucothoe stage for larvae fed PUFA enriched diets than larvae fed the control OA diet. Larvae that were fed diets enriched with DHA & EPA and DHA & AA had more and larger lipid droplets than larvae that received either the DHA or control (OA) diets. During the high mortality glaucothoe stage the total FAs per wet weight (µg/mg) was significantly lower in crabs fed the control diet. Further, during a salinity stress test glaucothoe previously fed the control OA diet recovered significantly slower than individuals previously fed PUFA-enriched diets, suggesting that PUFAs may improve performance during stress. These results advance our understanding of crustacean larval nutrition

and inform the feasibility of rehabilitating king crab stocks that crashed in the early 1980s throughout Alaska.

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Abbreviations

AA - Arachidonic acid (20:4n-6)

AKCRRAB - Alaska King Crab Research Rehabilitation and Biology

ANOVA – Analysis of variance

AWT – Ash weight

DHA - Docosahexaenoic acid (22:6n-3)

DWT – Dry weight

EFA – Essential fatty acids

EPA - Eicosapentaenoic acid (20:5n-3)

FA – Fatty acid

FFA – Free fatty acids

HUFA – Highly unsaturated fatty acids

MUFA – Monounsaturated fatty acids

PCA – Principal component analysis

PLs – Phospholipids

PUFA - Polyunsaturated fatty acids

RKC – Red king crab

SFA – Saturated fatty acids

STs - Sterols

TAGs – Triacylglycerols

WWT – Wet weight

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General Introduction

An understanding of potential bottlenecks in the life history of red king crab, *Paralithodes camtschaticus,* could improve fishery management and inform the potential for stock rehabilitation. Timing of reproduction varies slightly throughout their geographic range; from British Columbia northward throughout Alaska and the Bering Sea and southward along Asia to southwest of Korea (Stevens, 2014), but the overall cycle remains the same. Females molt annually and extrude 40,000 to 500,000 ova that are fertilized with sperm from a grasping male (Otto et al., 1990). Females then carry their embryos for approximately 300 days with hatching occurring in February and March. Red king crab (RKC) larvae develop through four zoeal stages followed by a non-feeding glaucothoe stage. Depending on temperature, zoeae molt to glaucothoe 21-30 days after hatching, and the glaucothoe stage lasts for 14-25 days, during which time crabs utilize endogenous energy before molting to the first juvenile stage (Kittaka et al., 2002; Swingle et al., 2013). During the glaucothoe stage, the mouthparts and entire digestive system of RKC larvae go through major morphological changes (Abrunhosa and Kittaka, 1997a; 1997b), which depletes lipid stores (Copeman et al., 2012; Stevens and Kittaka, 1998). Glaucothoe are excellent swimmers that seek suitable habitat as the crab undergoes metamorphosis into a benthic organism (Epelbaum et al., 2006; Stevens and Kittaka, 1998), but this stage can prove fatal if energy stores are exhausted (Stevens and Kittaka, 1998). As a result, the glaucothoe stage is a bottleneck both in the field and in the laboratory during their early life history. Survival during the four zoeal stages remains relatively high in the laboratory (50% to 74%), with survival during the glaucothoe stage ranging from 20% to 35% (Swingle et al., 2013). In the hatchery, mortality can vary year to year but the cause remains unknown (Swingle

et al., 2013). However, improvements to larval diets and assessment of larval condition at each developmental stage may provide insight into the sources of this mortality.

King crab is an important commercial fishery in Alaska (NPFMC, 2013). Fishing for Alaskan RKC by the US fleet extends back to the 1940s with commercial effort increasing in the Bering Sea in the late 1950s (Dew, 2010; Woodby et al., 2005). Specific hot spots for the fishery included Bristol Bay, Norton Sound, Petrel Bank, the Pribilof Islands, and Southeast Alaska (Woodby et al., 2005). However, by the early 1980s, many stocks were in decline or had collapsed. Red king crab fisheries in Prince William Sound, Cook Inlet, Kodiak, the Alaska Peninsula, and the Aleutians were closed in the mid-1980s and have failed to recover since then (Orensanz et al., 1998; Woodby et al., 2005). Obstacles potentially impeding recovery include climate change, overharvesting, and bycatch in other fisheries (Armstrong et al., 1993; Dew and McConnaughey, 2005; Woodby et al., 2005). Changes in climate may affect recruitment trends, as RKC stocks in the central and western Gulf of Alaska and Bristol Bay had good year classes during colder years in the early 1970s and lower year classes during warmer years in the late 1970s and early 1980s (Loher and Armstrong, 2005; Zheng and Kruse, 2000). Red king crab aggregate in deep and shallow waters depending on reproductive stage, which makes them easy to catch even when populations may be depleted (Taggart et al., 2008), thus making them particularly vulnerable to overharvesting and bycatch (Dew and McConnaughey, 2005). Despite efforts to reduce bycatch, injury and mortality still occur (Armstrong et al., 1993; Dew and McConnaughey, 2005). Since many collapsed RKC stocks have not recovered, and evidence suggests that they may be recruitment limited (Blau, 1986), stock rehabilitation may be a tool for their recovery.

The Alaska King Crab Research Rehabilitation and Biology (AKCRRAB) program has been investigating the feasibility of stock rehabilitation for Alaskan RKC and blue king crab (*P. platypus*) since 2006 (Daly et al., 2012). Their research has demonstrated that large-scale production could be highly efficient and successful in a hatchery setting (Daly et al., 2009). Larval survival increases when they are fed enriched *Artemia* along with other food sources, such as microalgae (Persselin and Daly, 2010). Increasing hatchery rearing success along with understanding the biological needs of wild RKC are key components of creating a successful stock rehabilitation program.

In order to increase production of king crab juveniles in hatcheries for wild release, research on dietary requirements during the larval stage is required. The present study focuses on the larval stages and dietary requirements of king crab raised in a hatchery. Chapter 1 reviews the literature on the importance of fatty acids (FAs) to the nutritional quality of enriched prey for use in culturing marine crustaceans. The objectives of this chapter are 1) to review the role of lipid, FAs and essential FAs (FAs that cannot be synthesized by animals in adequate amounts for normal growth and development and therefore must be acquired through their diet; EFAs) in larval crustacean diets and 2) to summarize studies that have examined impacts of EFAs on survival and growth of hatchery reared crustaceans. In Chapter 2, I compare survival, condition, and biochemistry of RKC larvae raised in a hatchery under four different diets in a live-feed experiment. The objectives of this chapter are to determine 1) the impacts of variable ratios of EFAs to the survival of RKC larvae, 2) the impacts of different ratios of dietary EFAs on larval recovery time from a stress test, 3) how lipid composition of the larvae changes across developmental stages when fed on differentially enriched diets, and 4) if dietary enrichments

impacts the ratios of EFA in king crab larvae. Based on the results, I provide recommendations on dietary EFA enrichment protocols for use in king crab hatcheries.

References

Abrunhosa, F.A., Kittaka, J., 1997a. Functional morphology of mouthparts and foregut of the last zoea, glaucothoe and first juvenile of the king crabs *Paralithodes camtschaticus*, *P. brevipes* and *P. platypus*. Fish. Sci. 63, 923-930.

Abrunhosa, F.A., Kittaka, J., 1997b. Morphological changes in the midgut, midgut gland, and hindgut during the larval and postlarval development of the red king crab *Paralithodes camtschaticus*. Fish. Sci. 63, 746-754.

Armstrong, D.A., Wainwright, T.C., Jensen, G.C., Dinnel, P.A., Andersen, H.B., 1993. Taking refuge from bycatch issues: red king crab (*Paralithodes camtschaticus*) and trawl fisheries in the Eastern Bering Sea. Can. J. Fish. Aquat. Sci. 50, 1993-2000.

Blau, S.F., 1986. Recent declines of red king crab (*Paralithodes camstchatica*) populations and reproductive conditions around the Kodiak Archipelago, Alaska. Can. Spec. Publ. Fish. Aquat. Sci. 92, 360-369.

Copeman, L.A., Stoner, A.W., Ottmar, M.L., Daly, B., Parrish, C.C., Eckert, G.L., 2012. Total lipids, lipid classes, and fatty acids of newly settled red king crab (*Paralithodes camtschaticus*): comparison of hatchery-cultured and wild crabs. J. Shellfish Res. 31, 153-165.

Daly, B., Swingle, J.S., Eckert, G.L., 2009. Effects of diet, stocking density, and substrate on survival and growth of hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles in Alaska, USA. Aquaculture. 293, 68-73.

Daly, B., Swingle, J.S., Eckert, G.L., 2012. Increasing hatchery production of juvenile red king crabs (*Paralithodes camtschaticus*) through size grading. Aquaculture. 364, 206-211.

Dew, C.B., 2010. Historical perspective on habitat essential to Bristol Bay red king crab. In: Kruse, G.H., Eckert, G.L., Foy, R.J., Lipicus, R.N., Sainte-Marie, B., Stram, D.L., Woodby, D. (Eds.), Biology and Management of Exploited Crab Populations Under Climate Change. Alaska Sea Grant, University of Alaska Fairbanks.

Dew, C.B., McConnaughey, R.A., 2005. Did trawling on the brood stock contribute to the collapse of Alaska's king crab? Ecol. Appl. 15, 919-941.

Epelbaum, A.B., Borisov, R.R., Kovatcheva, N.P., 2006. Early development of the red king crab (*Paralithodes camtschaticus)* from the Barents Sea reared under laboratory conditions: morphology and behaviour. J. Mar. Biol. Assoc. U.K. 86, 317-333.

Kittaka, J., Stevens, B.G., Teshima, S., Ishikawa, M., 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. In: Paul, A.J., Dawe, E.G., Elner, R., Jamieson, G.S., Kruse, G.H., Otto, R.S., Sainte-Marie, B., Shirley, T.C., Woodby, D. (Eds.), Crabs in Cold Water Regions: Biology, Management, and Economics.

Loher, T., Armstrong, D.A., 2005. Historical changes in the abundance and distribution of ovigerous red king crabs (*Paralithodes camtschaticus*) in Bristol Bay (Alaska), and potential relationship with bottom temperature. Fish. Oceanogr. 14, 292-306.

NPFMC, 2013. Stock assessment and fishery evaluation report for the king and tanner crab fisheries of the Bering sea and Aleutian islands regions. North Pacific Fishery Management Council, Anchorage, AK.

Orensanz, J.M.L., Armstrong, J., Armstrong, D., Hilborn, R., 1998. Crustacean resources are vulnerable to serial depletion: the multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. Rev. Fish Biol. Fish. 8, 117-176.

Otto, R., MacIntosh, R., Cummiskey, P., 1990. Fecundity and other reproductive parameters of female red king crab (*Paralithodes camtschatica*) in Bristol Bay and Norton Sound, Alaska, Proceedings of the international symposium on king and tanner crabs. Alaska Sea Grant college program report.

Persselin, S., Daly, B., 2010. Diet and water source effects on larval red king crab cultivation. In: Kruse, G.H., Eckert, G.L., Foy, R., Lipcius, R.N., Sainte-Marie, B., Stram, D.L., Woodby, D. (Eds.), Biology and Management of Exploited Crab Populations Under Climate Change. Alaska Sea Grant, University of Alaska Fairbanks.

Stevens, B.G., 2014. King crabs of the world: biology and fisheries management. CRC Press.

Stevens, B.G., Kittaka, J., 1998. Postlarval settling behavior, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus*. Mar. Ecol. Prog. Ser. 167, 197-206.

Swingle, J.S., Daly, B., Hetrick, J., 2013. Temperature effects on larval survival, larval period, and health of hatchery-reared red king crab, *Paralithodes camtschaticus*. Aquaculture. 384, 13-18.

Taggart, S.J., Mondragon, J., Andrews, A.G., Nielsen, J.K., 2008. Spatial patterns and movements of red king and Tanner crabs: implications for the design of marine protected areas. Mar. Ecol. Prog. Ser. 365, 151-163.

Woodby, D., Carlile, D., Siddeek, S., Funk, F., Clark, H.J., Hulbert, L., 2005. Commercial Fisheries of Alaska. Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services, Anchorage.

Zheng, J., Kruse, G.H., 2000. Recruitment patterns of Alaskan crabs in relation to decadal shifts in climate and physical oceanography. ICES J. Mar. Sci. 57, 438-451.

Chapter 1. A review of lipids and dietary fatty acid requirements for hatchery raised marine larval crustaceans[1]

1.1 Abstract

As the demand for seafood increases, aquaculture can help meet these needs while also enhancing or restoring depleted stocks. The goal of this review is to investigate the importance of using fatty acids (FAs) to enrich prey when culturing marine crustaceans for aquaculture. FAs play a vital role in the survival, growth, and stress tolerance of marine organisms. Although natural prey items are the ideal nutritional source, they are often hard to culture on a large scale for hatchery production. Therefore, other prey items, such as *Artemia* nauplii, are commonly used in crustacean aquaculture, because they are easy to culture in large amounts and are slow swimming. However, *Artemia* lack essential FAs (EFAs) required for normal growth and survival in crustaceans, and therefore require enrichment. Long-chain polyunsaturated FAs (PUFAs) are important in molting success, growth, ability to handle stress, and overall survival of crustaceans in a hatchery. Specifically, when the EFAs docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are added to the diet, the success of culturing crustaceans increases. Although different ratios of these FAs are required depending on the species, they have all shown to be important in larval survival and growth. Understanding the nutritional requirements of crustaceans is essential to increasing survival of larvae in a hatchery setting.

[1] Beder, A.M., Eckert, G.L., and Copeman, L.A. A review of lipids and dietary fatty acid requirements for hatchery raised marine larval crustaceans. Prepared for submission in Aquaculture

1.2 Introduction

The worldwide demand for fish and seafood supply has increased 3.2 percent each year from 1961 to 2009 (FAO, 2012). This increased demand puts pressure on the seafood industry and can deplete wild finfish and shellfish populations. Aquaculture and stock enhancement could be used to help meet this demand by enhancing or restoring depleted fisheries (Lorenzen et al., 2010). World aquaculture production increased in volume at an average rate of 8.6 percent a year between 1980 and 2012, with crustaceans comprising 9.7% of all aquaculture production in 2012 (FAO, 2014). These production statistics include hatchery production for wild stock enhancement. One of the bottlenecks to increasing hatchery production is nutrition, and as the diversity of species cultured increases, additional information is needed on the nutritional requirements of a wider variety of species. The goal of this review is to investigate the FA requirements of marine larval crustaceans in aquaculture.

1.3 Lipids

Lipids are an important energy source for marine organisms, making them a vital component of marine food webs (reviewed by Parrish, 2013). They are the densest form of energy, yielding two-thirds more energy per gram than protein or carbohydrates. A wide variety of lipid classes including triacylglycerols (TAGs), wax esters (WE), sterols (STs), and phospholipids (PLs) play important roles in marine organisms (Figure 1.1). Animals living in seasonal environments store energy as lipids when food is abundant. Some animals, such as copepods, crabs, and fish store large lipid droplets that can be visible under microscopes. Lipids are important in the structure and function of cell membranes and they help regulate membrane fluidity in cold or seasonal environments (Berge and Barnathan, 2005). Lipids are major sources of energy and are important for molting and growth success for juvenile and larval crustaceans

(Arts et al., 2001; Berge and Barnathan, 2005). Specifically, TAGs, STs, and PLs promote overall health of crustaceans (Coutteau et al., 1996; Wen et al., 2006) and make up over 80% of crustacean lipid pools (Coutteau et al., 1996; Ouellet et al., 1992).

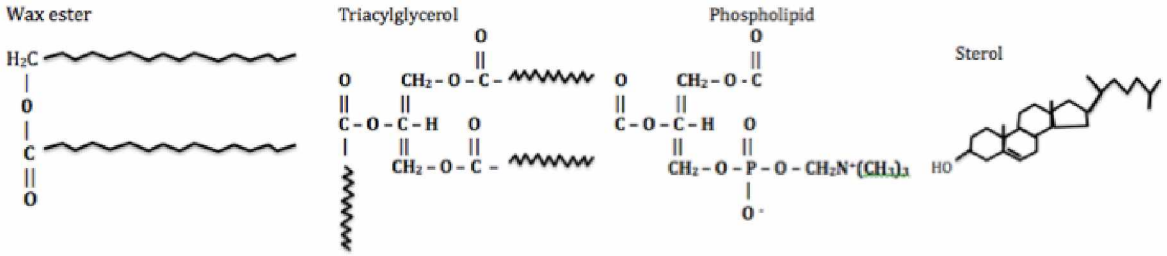

Figure 1.1. Major lipid classes found in marine crustaceans.

Lipids can be used for energy and can be stored as either WE, which consist of a long-chain fatty alcohol esterified with a FA, or TAGs, which consist of a glycerol molecule linked through ester bonds with three FAs (hydrocarbon chains with a carboxylic acid group) usually consisting of both saturated (no double bonds) and unsaturated (one or more double bonds) FAs (Champe et al., 2008). Wax esters are found in zooplankton and generally decrease in proportion relative to TAG in higher trophic levels. For example, copepods have both WE and TAG energy stores, while fish generally have only TAG (Coutteau et al., 1996; Ouellet et al., 1992). Triacylglycerol energy stores can sometimes be seen as oily droplets in larvae, juveniles, and adults of a wide variety of species of copepods (Hakanson, 1984). These energy stores are linked with successful growth and survival of crustaceans. Growth and molting success for Chinese mitten crabs (*Eriocheir sinensis*) is elevated with higher storage of TAG (Wen et al., 2006).

Accumulation of TAG in American lobsters during early larval stages increases survival during starvation (Sasaki, 1984; Sasaki et al., 1986) since TAG can be catabolized for energy.

Phospholipids are amphipathic compounds that comprise the structure of cell membranes and consist of a backbone of either glycerol or sphingosine (Wen et al., 2006). Organisms tend to conserve and convert dietary FAs into structural PL (Champe et al., 2008). Hemolymph in crustaceans contains large amounts of PL (Allen, 1972; Bligh and Scott, 1966; Huggins and Munday, 1968; Lee and Puppione, 1978). For example, hemolymph in spiny lobsters can contain up to 88% PL in the lipid content of hemolymph (Lee and Puppione, 1978). The percentage PL in hemolymph can change with temperature, as demonstrated in European green crab, *Carcinus maenas* (Brichon et al., 1980), which increases membrane fluidity.

Another important group of lipids are ST (Geurden et al., 1995; Kanazawa et al., 1985; Kanazawa et al., 1983; Radunz-Neto et al., 1994), which form important components of membranes and help to stabilize the phospholipid bilayer (Nes, 1974). Cholesterol, in particular, is the major ST found in animals and is primarily localized in the plasma membranes where it stabilizes cell structure and limits membrane permeability (Champe et al., 2008). Sterols serve as precursors of hormones, including ecdysteroids, in crustaceans (Spaziani et al., 1999). Many invertebrates, including crustaceans, are unable to synthesize cholesterol; therefore, they rely on dietary sources for somatic growth (Goad, 1981). Cholesterol serves as a precursor for the synthesis of molting hormones (Bloom and Mouritsen, 1988; Kroes and Ostwald, 1971) and is therefore a crucial component of crustacean diets. Phytosterols are synthesized by algae and plants (Hassett and Crockett, 2009) and are structurally similar to cholesterol. Phytosterols help to regulate membrane fluidity and are specifically desired in human food sources due to their benefits to human health, including lowering cholesterol and improving cardiovascular health

(Hassett and Crockett, 2009; Ostlund, 2002). However, crustaceans consist of more cholesterol than phytosterols (Phillips et al., 2012).

1.4 Fatty Acids

Fatty acids are major components of specific lipid classes such as TAG and PL (acyl lipid classes). The compositions of dietary FAs have important implications for crustacean growth and survival. Structurally, FAs are comprised of a carboxylic acid with a long aliphatic tail (chain), and in crabs the FA pool generally is comprised of chains with 14 to 24 carbons. FAs are classified into three different structural groups: saturated (SFA, contain no double bonds), monounsaturated (MUFA, contain one double bond), or polyunsaturated (PUFA, contain two to six double bonds). All FAs have a methyl terminus and a carboxylic acid terminus. As described in Champe et al. (2008), FAs are named using a shorthand notation of A:Bn-x, where A indicates the number of carbon atoms, B is the number of double bonds, and x references the position of the first double bond relative to the terminal methyl group.

Essential fatty acids (EFAs) cannot be synthesized in adequate amounts from dietary precursors, but they are required for normal growth and development and therefore must be acquired through food (Arts et al., 2001; Berge and Barnathan, 2005). Essential FAs can impact reproduction, immunity and buoyancy control (Glencross, 2009). Certain PUFAs with 20 chain carbon lengths (EPA, 20:5n-3 and AA, 20:4n-6) are EFAs that are important precursors of eicosanoids, also known as local hormones (Champe et al., 2008). Live foods used for culturing larvae, such as *Artemia* and nematodes, are naturally low in EFAs and therefore are often enriched before feeding to larvae (Lovern, 1934; Mjaavatten et al., 1998; Navarro et al., 1999).

Polyunsaturated FAs are biosynthesized by phytoplankton and microalgae and are bioaccumulated through the food chain (Brown et al., 1997; Parrish, 2009; Sargent et al., 1999;

Watanabe and Kiron, 1994). The importance of n-3 FAs has been investigated for a number of shrimp and crab species, including *Palaemon serratus* (Holme et al., 2007), *Homarus americanus* (Kamarudin and Roustaian, 2002), *Carcinus maenas* (Castell and Covey, 1976), and *Eriocheir sinensis* (Ponat and Adelung, 1983). When unenriched prey were fed to mud and swimming (*Portunus trituberculatus*) crab, juveniles showed prolonged molting, low survival, and reduced swimming activity compared to juveniles fed PUFA-enriched prey (Sheen and Wu, 1999; Takeuchi et al., 1999b). When commercial enrichments were used to enrich *Artemia* fed to spider crab (*Maja brachydactyla)* larvae, the enrichments increased PUFA levels in *Artemia* and resulted in increased survival and growth of the larvae compared to those fed unenriched *Artemia* (Andres et al., 2010).

Three essential PUFAs, docosahexaenoic (DHA, 22:6n-3), eicosapentaenoic (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are required for early growth and development of crustaceans (Andres et al., 2010; Coutteau et al., 1996). Microalgae contain essential PUFAs and are most rich in EPA and DHA (Brown et al., 1997). Docosahexaenoic acid is found at higher concentrations in dinoflagellates, whereas higher levels of EPA are generally associated with diatoms (Dunstan et al., 1994; Stevens et al., 2004). Arachidonic acid is found in macroalgae but is often in small proportions in marine plankton (Copeman and Parrish, 2003). Docosahexaenoic acid plays an important role in membrane structure and neural function (Takeuchi et al., 1999b). Eicosapentaenoic acid and AA are important components of membranes and are the substrates for the production of localized hormones (eicosanoids, prostaglandins and leukotrienes). These hormones play an important role in stress responses as well as other physiological processes such as gonad growth (Reddy et al., 2004; Sargent et al., 1995).

1.5 Enrichments of DHA, EPA and AA in Crustacean Larval Development

In order for aquaculture to be successful, prey items must be adequate in size, contain proper nutrition, and be able to survive in the hatchery setting (Beck and Turingan, 2007; McConaugha, 1985). Natural prey items, such as copepods, are nutritionally adequate for marine larvae (Peck and Holste, 2006; Sørensen et al., 2007); however, culturing natural prey is often difficult, unreliable, and often cannot be accomplished at sufficient densities (Figueiredo et al., 2009). Disease or parasites can also be introduced with natural prey items (Dhont et al., 2013). As an alternative, most hatcheries culture prey items such as *Artemia* nauplii and rotifers, because they can easily be raised at high densities and are slow swimming, which makes them suitable prey for a variety of organisms (Beck and Turingan, 2007; Narciso and Morais, 2001). Although easy to culture, *Artemia* nauplii and rotifers are naturally low in EFA compared to natural prey and are often enriched before feeding (Table 1.1; Fernandez-Reiriz et al., 1993; Figueiredo et al., 2009; Sorgeloos et al., 2001). Specifically, *Artemia* consist of low amounts of EPA, and DHA is practically absent, requiring enrichments of both EFAs (Suprayudi et al., 2004b; Takeuchi et al., 1999a). Nematodes, another common food source used in aquaculture, lack EFAs, especially DHA, and require enrichment if they are the sole food source (Honnens et al., 2014). Another way to enhance EFAs in cultured organisms is to feed them microalgae, but the culture of microalgae is labor-intensive. *Artemia* are most commonly used because they have a higher FA content, mostly AA and EPA, than algae and rotifers (Mourente et al., 1995). However, freshwater and saltwater *Artemia* cysts vary in FAs, depending on their source, with freshwater species containing higher levels of EPA, AA, and cholesterol, and salt water cysts containing a higher percentage of TAG (Navarro et al., 1993).

Table 1.1 Fatty acid profiles (% total FAs) of unenriched *Artemia* and copepods.

	DHA	EPA	AA	Source
Unenriched *Artemia*	0	2.4	Trace amounts	(Barclay and Zeller, 1996)
Copepods	6.0	34.0	0.0	(Copeman and Parrish, 2003)

Diets including DHA, EPA, and AA are required for survival and molting success of crustaceans. Higher percentages of EPA support larval molting (Sheen and Wu, 2002; Suprayudi et al., 2004a), and AA improves growth and molting success of larvae (Levine and Sulkin, 1984). Successful survival of spiny rock lobster (*Panulirus ornatus*) larvae requires food sources rich in AA, EPA, and DHA (Conlan et al., 2014). These same FA also increase survival of mud crab larvae during short-term starvation by providing energy stores that can be utilized (Holme et al., 2009). Higher survival in hatcheries is found for RKC larvae that are fed diatoms high in EPA (Kittaka et al., 2002). Lower survival, longer intermolt periods, and narrow carapace widths in lobsters, prawns, and shrimp have been caused by deficiencies in EPA and DHA (Castell and Covey, 1976; Kanazawa et al., 1979; Read, 1981).

Ratios of DHA:EPA can impact neural function and development in crustaceans. Fatty acids DHA and EPA compete against each other in esterification onto glycerol backbones (Parrish, 2013; Pirtle and Stoner, 2010). Neural tissue contains high amounts of DHA, which is thought to play a specific role in neural membrane structure and function (Bell and Dick, 1991). An elevated level of EPA compared to DHA can have a negative impact on neural function,

along with reducing growth and survival (Bell et al., 1995; Rodriguez et al., 1997). Developmental retardation and metamorphosis failure can occur when receiving higher ratios of DHA and EPA in mud crab (Suprayudi et al., 2004b). Providing adequate ratios of these two FAs improves crustaceans' condition.

The FAs EPA and AA are important precursors to localized hormones (eiconsanoids); however, EPA and AA can exhibit competitive interactions in hormone production. Of the two, AA produces hormones with higher biological activity (Kanazawa et al., 1985). Eicosanoids of lower biological activity are produced with EPA and high levels of EPA can restrict the use of AA in synthesizing these hormones. Required ratios can change depending on developmental stage (Holme et al., 2009). For example, RKC juveniles utilize lipids differently during postmolt, intermolt, and premolt stages with rapid accumulation occurring during the feeding-intermolt stage (Copeman et al., 2012). One way to determine the appropriate ratios to use in a hatchery is to compare wild and hatchery crustaceans. In a comparison of wild and hatchery RKC juveniles (2^{nd} juvenile molt, C2), the percentage of DHA did not differ, but wild crabs had higher proportions of AA and EPA than hatchery crabs (Table 1.2). The DHA:EPA ratio was higher in cultured (1.0) than wild (0.6) crabs. Comparisons such as this one can inform the formulation of appropriate diets in hatcheries.

Table 1.2. Percentage of major FAs in wild and cultured juvenile RKC.
Percentages (% total FAs) in stage 2 juveniles (C2) from Copeman et al. (2012). Data are the mean of 5 juveniles ± SD.

	DHA	EPA	AA
Wild	14.4 ± 1.4	24.7 ± 1.3	2.8 ± 0.3
Cultured	13.0 ± 0.4	13.3 ± 0.5	1.8 ± 0.1

1.6 Hatchery Stressors and Assessing Condition

Animals experience a number of stressors in a hatchery setting, including temperature, salinity, high densities, nutrition and ammonia, to name a few. Size, age, and molting stage can influence an individual's ability to handle different stressors (Briggs, 1992; Samocha et al., 1998). When giant tiger prawn (*Penaeus monodon fabricius)* were exposed to stressors, smaller and molting individuals had elevated mortality compared to older, larger individuals (Briggs, 1992). They also found that shrimp recover faster from stressors when receiving higher levels of n-3 PUFA (Fraser, 1989; Sasaki, 1984; Sasaki et al., 1986). Similar advantages of a highly unsaturated FA (long chain FA; HUFA) diet have been found for crabs, including Chinese mitten crabs, which are able to tolerate salinity stressors better than animals that received a HUFA limited diet (Chim et al., 2001; Palacios et al., 2004; Rees et al., 1994).

Being able to assess the condition of hatchery raised organisms is important, especially for outstocking purposes, to ensure the release of healthy individuals. Quick and effective methods of measuring relative amounts of lipid droplets in individual larvae are used for a number of cultured species to provide an index of larval condition (Anger and Hayd, 2009; Coutteau et al., 1996; Duguid and Page, 2009). Lipid droplets stored in the digestive gland have previously been assessed (size and number) to determine the condition of prawns,

Macrobrachium amazonicum, (Anger and Hayd, 2009) and RKC (Swingle et al., 2013). Visual quantification of lipids in the hatchery has been found to be an adequate means of quantifying condition in a variety of organisms (Angel-Dapa et al., 2010; Gallager and Mann, 1986b; Gallager et al., 1986; Rodriguez-Jaramillo et al., 2008).

A common condition assessment used on fish and crustaceans includes the ratio of TAG to ST (Fraser, 1989; Harding and Fraser, 1999). Excess energy can be stored as TAG and then later metabolized when an organism is unable to obtain sufficient energy from food (Ehrlich, 1974; Fraser et al., 1987). These TAG energy stores are important for survival for a number of juvenile and adult crustacean species (Heath and Barnes, 1970; New, 1976; Sasaki, 1984; Sasaki et al., 1986; Teshima et al., 1977). The ratio of TAG to ST is preferable to simply measuring TAG as a condition index, because storage of TAG is correlated with physiological condition of larvae and depends on larval size (Gallager and Mann, 1986a). Because ST levels are positively correlated with dry weight (Fraser et al., 1987), the ratio of TAG/ST is a size-standardized condition assessment (Fraser, 1989). The ratio of TAB/ST is preferred over TAG/PL, because ST levels are more stable during starvation than those of PL (Sasaki, 1984). Because TAG levels in crustaceans can decrease during molting, molting stage should be considered with using TAG/ST ratios to assess condition (Copeman et al., 2014).

1.7 Conclusion

As the demand for seafood has risen, so has larval culture of crustaceans for aquaculture. A number of crustacean species have been successfully raised in hatcheries. These successes help to meet the demand for seafood as well as provide the potential to restock depleted populations. Hatchery techniques vary depending on species, and a major component for success is diet. Fatty acids along with other lipids are important in diets of larval crustaceans.

Specifically EFAs can increase survival along with reducing hatchery stress. Three major EFAs needed in crustacean diets are DHA, EPA, and AA. Appropriate ratios of these FAs increase survival, ability to handle stress, and also condition hatchery animals for release into the wild. Dietary requirements are unique, so information from wild individuals can aid in developing EFA enrichments appropriate for a given species. Providing proper nutrition along with being able to assess condition can improve survival and condition of larvae raised in a hatchery.

1.8 References

Allen, W., 1972. Lipid transport in the Dungeness crab *Cancer magister* Dana. Comp. Biochem. Physiol., B. 43, 193-IN198.

Andres, M., Estevez, A., Hontoria, F., Rotllant, G., 2010. Differential utilization of biochemical components during larval development of the spider crab *Maja brachydactyla* (Decapoda: Majidae). Mar. Biol. 157, 2329-2340.

Angel-Dapa, M.A., Rodriguez-Jaramillo, C., Caceres-Martinez, C.J., Saucedo, P.E., 2010. Changes in lipid content of oocytes of the penshell *Atrina maura* as a criterion of gamete development and quality: a study of histochemistry and digital image analysis. J. Shellfish Res. 29, 407-413.

Anger, K., Hayd, L., 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquat. Bio. 7, 19-30.

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58, 122-137.

Barclay, W., Zeller, S., 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* nauplii by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc. 27, 314-322.

Beck, J., Turingan, R., 2007. The effects of zooplankton swimming behavior on prey-capture kinematics of red drum larvae, *Sciaenops ocellatus*. Mar. Biol. 151, 1463-1470.

Bell, M., Dick, J., 1991. Molecular species composition of the major diacyl glycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morhua*). Lipids. 26, 565-573.

Bell, M.V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary deficiency of docosahexaenoic acid impairs vision at low-light intensities in juvenile herring (*Clupea harengus* L.). Lipids. 30, 443-449.

Berge, J.P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In: Gal, Y.L., Ulber, R. (Eds.), Marine Biotechnology I, pp. 49-125.

Bligh, E., Scott, M.A., 1966. Blood lipids of the lobster, *Homarus americanus*. Journal of the Fisheries Board of Canada. 23, 1629-1631.

Bloom, M., Mouritsen, O.G., 1988. The evolution of membranes. Can. J. Chem. 66, 706-712.

Brichon, G., Chapelle, S., Zwingelstein, G., 1980. Phospholipids composition and metabolism in the hemolymph of *Carcinus maenas* (Crustacea, Decapoda)—effect of temperature. Comp. Biochem. Physiol., B. 67, 647-652.

Briggs, M.R.P., 1992. A stress test for determining vigour of post-larval *Penaeus monodon fabricius*. Aq. Fish. Manage. 23, 633-637.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture. 151, 315-331.

Castell, J.D., Covey, J.F., 1976. Dietary lipid requirements of adult lobsters, *Homarus americanus*. J. Nutr. 106, 1159-1165.

Champe, P.C., Harvey, R.A., Ferrier, D.R., 2008. Biochemistry, 4th ed. Wolters Kluwer Health/Lippincott Williams and Wilkins, Philadelphia, PA.

Chim, L., Lemaire, P., Delaporte, M., Le Moullac, G., Galois, R., Martin, J., 2001. Could a diet enriched with n- 3 highly unsaturated fatty acids be considered a promising way to enhance the immune defences and the resistance of penaeid prawns to environmental stress? Aquacult. Res. 32, 91-94.

Conlan, J.A., Jones, P.L., Turchini, G.M., Hall, M.R., Francis, D.S., 2014. Changes in the nutritional composition of captive early-mid stage *Panulirus ornatus* phyllosoma over ecdysis and larval development. Aquaculture. 434, 159-170.

Copeman, L., Daly, B., Eckert, G.L., Swingle, J., 2014. Storage and utilization of lipid classes and fatty acids during the early ontogeny of blue king crab, Paralithodes platypus. Aquaculture. 424, 86-94.

Copeman, L.A., Parrish, C.C., 2003. Marine lipids in a cold coastal ecosystem: Gilbert Bay, Labrador. Mar. Biol. 143, 1213-1227.

Copeman, L.A., Stoner, A.W., Ottmar, M.L., Daly, B., Parrish, C.C., Eckert, G.L., 2012. Total lipids, lipid classes, and fatty acids of newly settled red king crab (*Paralithodes camtschaticus*): comparison of hatchery-cultured and wild crabs. J. Shellfish Res. 31, 153-165.

Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus vannamei*. Aquaculture. 147, 261-273.

Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., Sorgeloos, P., 2013. Rotifers, *Artemia* and copepods as live feeds for fish larvae in aquaculture. Advances in Aquaculture Hatchery Technology, 157.

Duguid, W.D.P., Page, L.R., 2009. Larval and early post-larval morphology, growth, and behaviour of laboratory reared *Lopholithodes foraminatus* (brown box crab). J. Mar. Biol. Assoc. U.K. 89, 1607-1626.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.M., Jeffrey, S.W., 1994. Essential polyunsaturated fatty-acids from 14 species of diatom (*Bacillariophyceae*). Phytochemistry. 35, 155-161.

Ehrlich, K., 1974. Chemical changes during growth and starvation of larval *Pleuronectes platessa*. Mar. Biol. 24, 39-48.

FAO, 2012. The state of the world fisheries and aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2014. The state of the world fisheries and aquaculture 2014. Food and Agriculture Organization of the United Nations, Rome.

Fernandez-Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional-value of the rotifer (*Brachionus-plicatilis*). Aquaculture. 112, 195-206.

Figueiredo, J., van Woesik, R., Lin, J., Narciso, L., 2009. *Artemia* franciscana enrichment model - how to keep them small, rich and alive? Aquaculture. 294, 212-220.

Fraser, A., Sargent, J., Gamble, J.C., MacLachlan, P., 1987. Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. American Fishery Society Symposium. 2, 129-143.

Fraser, A.J., 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. J. Fish. Aquat. Sci. 46, 1868-1873.

Gallager, S.M., Mann, R., 1986a. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. Aquaculture. 56, 105-121.

Gallager, S.M., Mann, R., 1986b. Growth and survival of larvae of *Mercenaria mercenaria* (L) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid-content of eggs. Aquaculture. 56, 105-121.

Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture. 56, 81-103.

Geurden, I., Radunzneto, J., Bergot, P., 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. Aquaculture. 131, 303-314.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquacult. 1, 71-124.

Goad, L.J., 1981. Sterol biosynthesis and metabolism in marine invertebrates. Pure App. Chem. 53, 837-852.

Hakanson, J.L., 1984. The long and short-term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid-content. Limnol. Oceanogr. 29, 794-804.

Harding, G.C., Fraser, A.J., 1999. Application of the triacylglycerol/sterol condition index to the interpretation of larval lobster *Homarus americanus* distribution in close proximity to Georges Bank, Gulf of Maine. Mar. Ecol. Prog. Ser. 186, 239-254.

Hassett, R.P., Crockett, E.L., 2009. Habitat temperature is an important determinant of cholesterol contents in copepods. J. Exp. Biol. 212, 71-77.

Heath, J.R., Barnes, H., 1970. Some changes in biochemical composition with season and during the molting cycle of the common shore crab *Carcinus maenas* (L). J. Exp. Mar. Biol. Ecol. 5, 199-223.

Holme, M.-H., Southgate, P.C., Zeng, C., 2007. Survival, development and growth response of mud crab, *Scylla serrata*, megalopae fed semi-purified diets containing various fish oil : corn oil ratios. Aquaculture. 269, 427-435.

Holme, M.H., Brock, I., Southgate, P.C., Zeng, C.S., 2009. Effects of starvation and feeding on lipid class and fatty acid profile of late stage mud crab, *Scylla serrata*, larvae. J. World Aquacult. Soc. 40, 493-504.

Honnens, H., Assheuer, T., Ehlers, R.U., 2014. Enrichment of the nematode *Panagrolaimus sp.*, a potential live food for marine aquaculture, with essential n-3 fatty acids. Aquacult. Int. 22, 399-409.

Huggins, A., Munday, K., 1968. Crustacean metabolism., Advances in Comparative Physiology and Biochemistry. Academic Press New York, pp. 271-378.

Kamarudin, M., Roustaian, P., 2002. Growth and fatty acid composition of freshwater prawn, *Macrobrachium rosenbergii*, larvae fed diets containing various ratios of cod liver oil–corn oil mixture. J. Appl. Ichthyol. 18, 148-153.

Kanazawa, A., Teshima, S.I., Sakamoto, M., 1985. Effects of dietary lipids, fatty-acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture. 50, 39-49.

Kanazawa, A., Teshima, S., Tokiwa, S., Ceccaldi, H., 1979. Effects of dietary linoleic and linolenic acids on growth of prawn. Oceanol. Acta. 2, 41-48.

Kanazawa, A., Teshima, S., Kobayshi, T., Takae, M., Iwashita, T., Uehara, R., 1983. Necessity of dietary phospholipids for growth of the larval Ayu. Mem. Fac. Fish., Kagoshima Univ. 32, 115-120.

Kittaka, J., Stevens, B.G., Teshima, S., Ishikawa, M., 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. In: Paul, A.J., Dawe, E.G., Elner, R., Jamieson, G.S., Kruse, G.H., Otto, R.S., Sainte-Marie, B., Shirley, T.C., Woodby, D. (Eds.), Crabs in Cold Water Regions: Biology, Management, and Economics.

Kroes, J., Ostwald, R., 1971. Erythrocyte membranes effect of increased cholesterol content on permeability. Biochim. Biophys. Acta Biochim Biophys Acta. 249, 647-650.

Lee, R.F., Puppione, D.L., 1978. Serum lipoproteins in the spiny lobster, *Panulirus interruptus*. Comp. Biochem. Physiol., B. 59, 239-243.

Levine, D.M., Sulkin, S.D., 1984. Nutritional significance of long-chain poly-unsaturated fatty-acids to the zoeal development of the Brachyuran crab, *Eurypanopeus depressus* (Smith). J. Exp. Mar. Biol. Ecol. 81, 211-223.

Lorenzen, K., Leber, K.M., Blankenship, H.L., 2010. Responsible approach to marine stock enhancement: an update. Rev. Fish. Sci. 18, 189-210.

Lovern, J.A., 1934. CCLVI. Fat metabolism in fishes v. the fat of the salmon in its young freshwater stages. Biochem. J. 29, 1961-1963.

McConaugha, J.R., 1985. Nutrition and larval growth. In: Wenner, A.M. (Ed.), Larval Growth - Crustacean Issues. AA Balkema Publishers, Netherlands, pp. 127-154.

Mjaavatten, O., Levings, C.D., Poon, P., 1998. Variation in the fatty acid composition of juvenile chinook and coho salmon from Fraser river estuary determined by multivariate analysis; role of environment, and genetic origin. Comp. Biochem. Physiol., B. 120, 291-309.

Mourente, G., Medina, A., Gonzalez, S., Rodriguez, A., 1995. Variations in lipid-content and nutritional-status during larval development of the marine shrimp *Penaeus-kerathurus*. Aquaculture. 130, 187-199.

Narciso, L., Morais, S., 2001. Fatty acid profile of *Palaemon serratus* (Palaemonidae) eggs and larvae during embryonic and larval development using different live diets. J. Crust. Biol. 21, 566-574.

Navarro, J.C., Amat, F., Sargent, J.R., 1993. The lipids of the cysts of fresh-water-type and marine-type *Artemia*. Aquaculture. 109, 327-336.

Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V., Amat, F., 1999. Lipid conversions during enrichment of *Artemia*. Aquaculture. 174, 155-166.

Nes, W.R., 1974. Role of sterols in membranes. Lipids. 9, 596-612.

New, M.B., 1976. A review of dietary studies with shrimp and prawns. Aquaculture. 9, 101-144.

Ostlund, R.E., 2002. Phytosterols in human nutrition. Annu. Rev. Nutr. 22, 533-549.

Ouellet, P., Taggart, C.T., Frank, K.T., 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. Can. J. Fish. Aquat. Sci. 49, 368-378.

Palacios, E., Bonilla, A., Pérez, A., Racotta, I.S., Civera, R., 2004. Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) postlarvae to low salinity. J. Exp. Mar. Biol. Ecol. 299, 201-215.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, Dordrecht, pp. 309-326.

Parrish, C.C., 2013. Lipids in marine ecosystems. ISRN Oceanography. 2013, 16.

Peck, M.A., Holste, L., 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures. Aquaculture. 255, 341-350.

Phillips, K.M., Ruggio, D.M., Exler, J., Patterson, K.Y., 2012. Sterol composition of shellfish species commonly consumed in the United States. Food & nutrition research. 56.

Pirtle, J.L., Stoner, A.W., 2010. Red king crab (*Paralithodes camtschaticus*) early post-settlement habitat choice: structure, food, and ontogeny. J. Exp. Mar. Biol. Ecol. 393, 130-137.

Ponat, A., Adelung, D., 1983. Studies to establish an optimal diet for *Carcinus maenas*. Mar. Biol. 74, 275-279.

Radunz-Neto, J., Corraze, G., Charlon, N., Bergot, P., 1994. Lipid supplementation of casein-based purified diets for carp (*Cyprinus carpio* L) larvae. Aquaculture. 128, 153-161.

Read, G., 1981. The response of *Penaeus indicus* (Crustacea: Penaeidea) to purified and compounded diets of varying fatty acid composition. Aquaculture. 24, 245-256.

Reddy, P.S., Reddy, P.R., Nagaraju, G.P.C., 2004. The synthesis and effects of prostaglandins on the ovary of the crab *Oziotelphusa senex senex*. Gen. Comp. Endocrinol. 135, 35-41.

Rees, J.-F., Curé, K., Piyatiratitivorakul, S., Sorgeloos, P., Menasveta, P., 1994. Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae: an experimental approach based on *Artemia* enrichment. Aquaculture. 122, 193-207.

Rodriguez, C., Perez, J.A., Diaz, M., Izquierdo, M.S., Fernandez-Palacios, H., Lorenzo, A., 1997. Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. Aquaculture. 150, 77-89.

Rodriguez-Jaramillo, C., Hurtado, M.A., Romero-Vivas, E., Ramirez, J.L., Manzano, M., Palacios, E., 2008. Gonadal development and histochemistry of the tropical oyster, *Crassostrea corteziensis* (Hertlein, 1951) during an annual reproductive cycle. J. Shellfish Res. 27, 1129-1141.

Samocha, T.M., Guajardo, H., Lawrence, A.L., Castille, F.L., Speed, M., McKee, D.A., Page, K.I., 1998. A simple stress test for *Penaeus vannamei* postlarvae. Aquaculture. 165, 233-242.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture. 177, 191-199.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183-198.

Sasaki, G.C., 1984. Biochemical changes associated with embryonic and larval development in the American lobster *Homarus americanus* Milne Edwards. Massachusetts Institute of Technology and Woods Hole Oceanographic Institution.

Sasaki, G.C., Capuzzo, J.M., Biesiot, P., 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. Can. J. Fish. Aquat. Sci. 43, 2311-2319.

Sheen, S., Wu, S., 2002. Essential fatty acid requirements of juvenile mud crab, *Scylla serrata* (Forskål, 1775)(Decapoda, scyllaridae). Crustaceana. 75, 1387-1401.

Sheen, S.S., Wu, S.W., 1999. The effects of dietary lipid levels on the growth response of juvenile mud crab *Scylla serrata*. Aquaculture. 175, 143-153.

Sørensen, T.F., Drillet, G., Engell-Sørensen, K., Hansen, B.W., Ramløv, H., 2007. Production and biochemical composition of eggs from neritic calanoid copepods reared in large outdoor tanks (Limfjord, Denmark). Aquaculture. 263, 84-96.

Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture. 200, 147-159.

Spaziani, E., Mattson, M.P., Wang, W.N.L., McDougall, H.E., 1999. Signaling pathways for ecdysteroid hormone synthesis in crustacean Y-organs. Am. Zool. 39, 496-512.

Stevens, C.J., Deibel, D., Parrish, C.C., 2004. Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). Mar. Biol. 144, 905-915.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K., 2004a. Essential fatty acids for larval mud crab *Scylla serrata*: implications of lack of the ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. Aquaculture. 231, 403-416.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K., 2004b. Effects of *Artemia* enriched with eicosapentaenoic and docosahexaenoic acid on survival and occurrence of molting failure in megalop larvae of the mud crab *Scylla serrata*. Fish. Sci. 70, 650-658.

Swingle, J.S., Daly, B., Hetrick, J., 2013. Temperature effects on larval survival, larval period, and health of hatchery-reared red king crab, *Paralithodes camtschaticus*. Aquaculture. 384, 13-18.

Takeuchi, T., Nakamoto, Y., Hamasaki, K., Sekiya, S., Watanabe, T., 1999a. Requirement of n-3 highly unsaturated fatty acids for larval swimming crab *Portunus trituberculatus*. Nippon Suisan Gakkaishi. 65, 797-803.

Takeuchi, T., Satoh, N., Sekiya, S., Shimizu, T., Watanabe, T., 1999b. The effect of dietary EPA and DHA on the molting rate of larval swimming crab *Portunus trituberculatus*. Nippon Suisan Gakkaishi. 65, 998-1004.

Teshima, S., Kanazawa, A., Okamoto, H., 1977. Variation in lipid classes during the molting cycle of the prawn *Penaeus japonicus*. Mar. Biol. 39, 129-136.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture. 124, 223-251.

Wen, X.B., Chen, L.Q., Ku, Y.M., Zhou, K.Y., 2006. Effect of feeding and lack of food on the growth, gross biochemical and fatty acid composition of juvenile crab, *Eriocheir sinensis*. Aquaculture. 252, 598-607.

Chapter 2. The effects of dietary live-food essential fatty acid ratios on the condition, stress response and survival of red king crab larvae (*Paralithodes camtschaticus*)[1]

2.1 Abstract

Traditional live foods used in aquaculture are convenient but often have low levels of essential fatty acids (EFAs), resulting in poor nutritional quality. I conducted a live-food enrichment experiment to investigate the effects of dietary EFAs on the growth, survival, and energy storage of red king crab (*Paralithodes camtschaticus)* larvae raised in a hatchery. *Artemia* nauplii that were fed to king crab larvae were enriched with four combinations of fatty acids (FAs), including 1) docosahexaenoic acid (DHA, 22:6n-3), 2) DHA and eicosapentaenoic acid (EPA, 20:5n-3), 3) DHA and arachidonic acid (AA, 20:4n-6), and 4) a control diet that was enriched with oleic acid (OA, 18:1n-9). The proportions of EFAs were significantly different in crabs from all dietary treatments during the fourth zoeal, glaucothoe, and first juvenile stages. During the high mortality glaucothoe stage the total FAs per WWT (µg/mg) were significantly lower in larvae fed the control diet. Larval condition, assessed by viewing size and number of lipid droplets, was significantly different among the four diet treatments at the glaucothoe stage. Larvae previously fed diets enriched with DHA & EPA and DHA & AA had more and larger lipid droplets than larvae that received either the DHA alone or control (OA) diets. Further, during a salinity stress test glaucothoe previously fed the control OA diet recovered significantly slower than individuals fed EFA enriched diets, suggesting that EFAs improve performance during stress. These results advance our understanding of crustacean larval nutrition to improve

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[1] Beder, A.M., Eckert, G.L., and Copeman, L.A. The effects of dietary live-food essential fatty acid ratios on the condition, stress response and survival of red king crab larvae (*Paralithodes camtschaticus*). Prepared for submission in Aquaculture

live-food dietary enrichment during the hatchery stage of the crab stock enhancement endeavor. Improved larval nutrition is vital to the mass production of juveniles for outstocking and rehabilitation of king crab stocks that originally crashed in Alaska during the early 1980s.

2.2 Introduction

Red king crab (*Paralithodes camtschaticus*) is an important commercial fishery in Alaska; however, since the early 1980s many stocks have been depleted and are closed to fishing (NPFMC, 2013; Orensanz et al., 1998). Despite several decades of fishing moratoria, many king crab stocks have not recovered, and therefore, juvenile crab culture and outplanting has been embraced as a potential means of stock rehabilitation. The Alaska King Crab Research Rehabilitation and Biology (AKCRRAB) program has been investigating the feasibility of stock rehabilitation for Alaskan RKC and blue king crab (*Paralithodes platypus*) since 2006 (Daly et al., 2012). Crustacean aquaculture has been successful for a number of other decapod crustaceans around the world, including European lobster (*Homarus gammarus*), Chesapeake Bay blue crab (*Callinectes sapidus*), mud crabs (*Scylla paramamosain*), and penaeid prawns (Agnalt, 2008; Bannister and Addison, 1998; Hamasaki and Kitada, 2008; Zohar et al., 2008).

Large-scale production of king crab in Alaska is successful in a hatchery setting (Daly et al., 2009). Advances in hatchery survival were obtained by increasing water temperature to speed up molt frequency and decrease development time, thus reducing detrimental effects of filamentous bacteria (Swingle et al., 2013). Survival also increased when larvae were fed *Artemia* along with other food sources, such as microalgae with *Artemia* (Persselin and Daly, 2010). Juvenile survival increased and cannibalism decreased when substrate was added to the tanks; this was likely observed because structure reduced the antagonistic interactions between

conspecifics in the same tank (Daly et al., 2009; Stoner et al., 2010). Increasing husbandry practices along with understanding the nutritional requirements of RKC are key components of creating a successful stock rehabilitation program.

An adequate diet is essential to rearing crustacean larvae in a hatchery setting and is specifically important for larval survival during the non-feeding glaucothoe stage, when crabs depend on nutrients stored previously during the feeding zoeal stages (Epelbaum and Kovatcheva, 2005). Red king crabs undergo four zoeal larval stages followed by one glaucothoe stage (as described in Daly et al., 2009; Epelbaum et al., 2006), During the glaucothoe stage, the mouthparts and entire digestive system of king crab larvae go through major morphological changes (Abrunhosa and Kittaka, 1997a; 1997b), and this stage can prove fatal if energy stores are insufficient (Copeman et al., 2012; Stevens and Kittaka, 1998). As a result, the glaucothoe stage is a critical period in hatchery production, with mass mortality occurring during this period. During past efforts, survival during the four zoeal stages (ZI to ZIV) was relatively high (50% to 74%), with survival from the glaucothoe (G) stage to the first crab stage (C1) ranging from approximately 20% to 35% (Swingle et al., 2013). This mortality can vary year to year, and although the cause remains unknown, one source of mortality could be diet-related.

Lipids are an important energy source for marine organisms, making them a vital component of marine food webs (Arts et al., 2001; Berge and Barnathan, 2005). They are the densest form of energy, yielding two-thirds more energy per gram than protein or carbohydrates (as described in Parrish, 2013). For juvenile and larval crustaceans, dietary lipids are important factors in improving growth and molting success (Glencross, 2009; Tziouveli and Smith, 2012; Wen et al., 2006; Xu et al., 1994). The major lipid classes in crustacean tissues are triacylglycerols (TAGs), sterols (STs), and phospholipids (PLs). Energy can be stored as TAGs

and are often seen as oily droplets in larvae (Hakanson, 1984). Phospholipids are the major structural compound in cell membranes (Champe et al., 2008) and are also important in antioxidant functions and buoyancy control (Coutteau et al., 1997). The PL bilayer in cell membranes is stabilized by STs (Champe et al., 2008), with cholesterol being the main ST in crabs. Crustaceans are unable to synthesize cholesterol (Goad, 1981) and rely on dietary sources for somatic growth and reproduction (Hassett and Crockett, 2009). Both absolute and relative amounts of these lipid classes can reflect the health and condition of crustaceans (Fraser, 1989; Harding and Fraser, 1999).

Essential FAs are acquired through diet and are essential for larval survival in crustaceans. Numerous studies have investigated the impacts of EFAs on larval survival, growth, and condition (Limbourn and Nichols, 2009; Suprayudi et al., 2004b; Takeuchi et al., 1999; Zmora et al., 2005). Specific dietary PUFAs are required in juvenile crab diets (Sheen and Wu, 2002). In marine environments, PUFAs are biosynthesized by phytoplankton and microalgae and are concentrated with increasing trophic levels (as described in Budge et al., 2006). Membrane fluidity is influenced by the composition of PUFAs within the PL lipid class, with increasing fluidity correlated to higher chain lengths and numbers of double bonds (Parrish, 2009; Sargent et al., 1999b; Watanabe and Kiron, 1994). Therefore, high proportions of long chain (C_{20+22}) highly unstaturated FAs (HUFA) are required for successful crustacean larval development and molting at cold-temperatures (Andres et al., 2010; Coutteau et al., 1997).

Three essential HUFAs, docosahexaenoic (DHA, 22:6n-3), eicosapentaenoic (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are required in the early growth and development of crustaceans (Andres et al., 2010; Coutteau et al., 1996). Microalgae are an important source of HUFAs, as DHA is found in high concentrations in dinoflagellates, and high concentrations of

EPA are generally associated with diatoms (Dunstan et al., 1994; Stevens et al., 2004). Arachidonic acid is found in macroalgae but often in small proportions in marine plankton (Figueiredo et al., 2009; Navarro et al., 1999; Sorgeloos et al., 2001). Live-foods used in commercial larviculture such as *Artemia* and rotifers (*Brachionus* sp.) are naturally very low in HUFA. For example, there are low amounts of EPA (2.4%) in *Artemia* and DHA is practically absent, requiring enrichments of live-food with lipids rich in HUFA prior to larval feeding (Barclay and Zeller, 1996; Fernandez-Reiriz et al., 1993; McEvoy et al., 1996; Navarro et al., 1999). *Artemia* are a difficult live-food to enrich because as they assimilate DHA they retro-convert DHA to EPA (Sargent et al., 1999a) and reduce the levels of DHA available for crab larvae. Therefore, quick feeding of live-food following enrichment is desirable (Cutts et al., 2006).

Few nutrition experiments have been previously performed on cold-water crab larvae from the genus *Paralithodes* (Daly et al., 2013; Epelbaum and Kovatcheva, 2005; Stevens, 2012; Stevens et al., 2008). However, a previous study by Copeman et al. (2014) showed that total lipids and proportions of TAGs increase in RKC larvae during successive larval stages and then decrease during the non-feeding glaucothoe stage (Copeman et al., 2014). Measurement of lipid class and FAs during the non-feeding glaucothoe stage revealed that C_{20+22} HUFAs are retained and increased proportionally during this starvation period. It was postulated that retention of these HUFA was because of their essential role in early growth, development and survival (Copeman et al., 2014). When comparing the same age (C2) wild crabs to hatchery raised king crabs, higher proportions of EPA and AA were found in wild crabs (Copeman et al., 2012), indicating that if "nature knows best," higher proportions of these two C_{20} HUFA should be added to live-food.

This study aimed to determine if growth, survival, and condition of RKC larvae were influenced by diets enriched with different ratios of DHA, EPA, and AA. Currently, Alaskan RKC larvae are raised on a diet of San Francisco Bay strain *Artemia* nauplii that are enriched using DC DHA Selco (INVE Aquaculture, UT, USA), a common enrichment emulsion used in aquaculture (Copeman et al., 2012; Swingle et al., 2013). Improvements to larval diets and assessment of larval condition at each molt stage may provide insight into the high mortality that is known to occur during the glaucothoe stage. Specifically, this study aimed to answer the following four questions for the early ontogeny of RKC larvae: 1) do different dietary ratios of HUFAs impact crab survival, 2) do different dietary ratios of HUFAs impact crab recovery time from a salinity stress test, 3) do different dietary ratios of HUFAs impact a visual condition index, 4) do different ratios of dietary HUFA impact the lipid class composition and fatty acid composition of crab across multiple developmental stages?

2.3 Materials and Methods

2.3.1 Broodstock and Larval Husbandry

Twelve ovigerous females (broodstock) were captured with pots near Juneau, Alaska in October 2012 during the Alaska Department of Fish and Game Southeast Alaska Tanner crab survey. They were maintained at the University of Alaska Fairbanks Juneau Center of the School of Fisheries and Ocean Sciences and transported to the UAF Marine Science Center in Seward, Alaska in March 2013. Broodstock were maintained in flow-through seawater under ambient conditions and fed herring *ad libitum* until embryos began hatching. At this time, broodstock were transferred to the Alutiiq Pride Shellfish Hatchery (APSH) and placed into individual cylindrical hatching bins (0.58 m diameter, 0.58 m deep) with 100 μm mesh bottoms. Over a two-day period, twelve 190 L tanks were stocked with 114,000 actively swimming larvae mixed

from eight females. Larval tanks were aerated and continuously received heated (9 ± 0.6 °C) seawater at a rate of 1 L per minute.

2.3.2 Larval Nutrition Experiment

Larvae were fed San Francisco Bay strain *Artemia* nauplii that were hatched for 24 h and then enriched in five-gallon enrichment buckets for another 24 h. Four experimental emulsions were used to enrich *Artemia*, a control (olive oil, oleic acid, OA), DHA, DHA & EPA, and DHA & AA (Table 2.1). These treatments were chosen with the goal of generating the proportions of EFAs found in wild juvenile crabs (Copeman et al., 2012). Larvae were fed twice a day (0700 h and 1700 h) with 1, 1.5, 1.75 and 2 *Artemia* per ml for the ZI, ZII, ZIII, and ZIV stages respectively, to insure larvae were being fed to satiation. A 105 μm screen was placed in the tank every morning before feeding and switched out with a 500 μm screen before the evening feeding to allow larvae to feed during the day, while flushing waste and excess food throughout the night. Culture tanks were not checked for remaining *Artemia* since past studies showed that changing the screens in the evenings completely flushes tanks (Daly et al., 2012; 2013; Swingle et al., 2013).

2.3.3 Survival and Visual Condition Assessment

Survival was estimated for each tank at mid Z2, mid Z3, and mid Z4 using volumetric sampling, where crabs were counted from five 1L subsamples collected from a fully-aerated and mixed tank. The average count was then multiplied by the total volume of the tank for the survival estimate. Tanks were drained for cleaning at the beginning of the glaucothoe and juvenile stages, and the 1L glaucothoe and juvenile subsamples were collected from a concentrated 50L volume. Visual condition assessments were conducted at stocking, two to three days before molting (intermolt) to each larval stage, and at the early glaucothoe stage. Tanks

were mixed using increased aeration and ten larvae were collected haphazardly from each tank. The number of lipid droplets in each larva was counted at 40x magnification, and the maximum lipid diameter measured at 100x magnification (both to the nearest μm).

Table 2.1. Biochemical composition of the enrichment emulsions (average ± SE, n=3).

Lipid parameters	Control (olive oil)	DHA	DHA & EPA	DHA & AA
Total fatty acids (μg/mg WWT)	16.4 ± 1.6	19.2 ± 1.7	19.9 ± 1.8	18.4 ± 0.6
% total fatty acids				
DHA	0.0 ± 0.0	17.0 ± 1.6	9.3 ± 0.6	11.5 ± 1.0
EPA	0.9 ± 0.1	3.6 ± 0.1	5.8 ± 0.6	3.0 ± 0.0
AA	0.3 ± 0.0	0.2 ± 0.1	0.5 ± 0.0	7.1 ± 0.0

2.3.4 Stress Test

In order to determine how diet affects larval response to stress, I conducted a modified freshwater stress test, similar to those conducted on *Penaeus* shrimp (Palacios et al., 2004). Twenty glaucothoe from each rearing tank were transferred to a Petri dish with distilled water for five minutes and then put into a petri dish with seawater. To minimize change in temperature, distilled water and seawater were maintained in containers in the larval rearing tanks until the experiment started, after which temperature was not controlled. Time was measured from when glaucothoe were first placed in the seawater until they "recovered". Recovery was defined as a crab traveling at least one body length. The experiment was completed when ten glaucothoe recovered. The first ten crabs to recover were considered fast recovery and the remaining ten

slow recovery. After the stress test, a visual condition assessment was conducted on the glaucothoe from the two petri dishes before glaucothoe were frozen for biochemical analyses.

2.3.5 Weights and Biochemistry

Larval samples for biochemical analyses were collected within eight hours of hatching (early Z1), at mid Z4, from glaucothoe used in the stress tests, and three days after molting to C1. Samples of *Artemia* were collected around the beginning, middle, and end of the experiment to confirm enrichment biochemistry. All samples were frozen at -20 °C, shipped on dry ice, stored at -80 °C, and analyzed at the Marine Lipids Laboratory, Cooperative Institute for Marine Resources Studies, Oregon State University, Hatfield Marine Sciences Center in Newport, Oregon. Larvae and crabs were weighed with a microbalance (Sartorius R160P) to the nearest µg to obtain a wet weight (WWT), dried for 48 h at 70 °C, and reweighed to get dry weight (DWT). Ash weight (AWT) was obtained by combusting crabs in a Barnstead Thermolyne furnace for 8 h at 450 °C and then reweighing. Pooled samples were weighed for each tank, with pools of ten larvae during the Z1 and Z4 stages, and two at the glaucothoe stage. Due to considerable weight variability during the juvenile stage, weights were averaged for three pooled samples from each tank, including two individuals in the first pool and five juveniles in the second and third pool.

Lipids were extracted in chloroform-methanol-water (5:4:1) according to Parrish (1987) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using a MARK V Iatroscan (Iatron Laboratories, Inc., Tokyo, Japan) as described by Lu et al. (2008) and Copeman et al. (2014). Chromarods were spotted with crab extracts (2 µl), and each sample was run in duplicate to reduce analytical error. Chromarods were developed in a chloroform:methanol:water solution (5:4:1 by volume) until the leading edge of the solvent

reached 1 cm above the origin. These rods were developed in a hexane:diethyl ether:formic acid solution (99:1:0.05 by volume) for 48 minutes followed by a 38 minute development in a hexane:diethyl ether:formic acid solution (80:20:0.1 by volume). This allowed the separation of lipid peaks from the most abundant lipid classes (TAG, ST and PL). After each development, rods were air-dried for five minutes and then conditioned for 5 minutes in a constant humidity chamber (~30%) that was saturated with aqueous $CaCl_2$. After the final development system, rods were scanned and the eluted peaks were analyzed using Peak Simple (version 3.67, SRI Inc.). Concentrations of lipids were quantified against lipid standards (Sigma, St Louis, MO, USA).

Lipid extracts were examined to determine fatty acid composition. Fatty acid methyl esters (FAMEs) were prepared by transesterification with 1.5 ml of methylene chloride and 3 ml Hilditch reagent (1.5 ml sulfuric acid, H_2SO4, in 100 ml methanol) before oven drying for 1 h at 100 °C. A HP 6890 capillary gas chromatographer (GC) flame ionization detector (FID) with an autosampler and a DB wax+ GC column (Agilent Technologies, Inc., U.S.A.) was used to analyze the FAMEs. The GC column was 30 m in length, with an internal diameter of 0.32 μm. The initial column temperature was maintained at 65 °C for 0.5 minutes, increased to 195 °C (40 °C min^{-1}) for 15 minutes, and then increased again (2 °C min^{-1}) to a final temperature of 220 °C for 3.25 minutes. The injector and detector temperatures were both at 260 °C. Peaks were identified using retention times based upon standards purchased from Supelco (37 component FAME, BAME, PUFA 1, PUFA 3). A known amount of 23:0 was added as an internal standard while, Nu-Check Prep GLC 487 quantitative FA mixed standard was used to develop correction factors for individual FAs. Chromatograms were integrated using Chem Station (version A.01.02, Agilent).

2.3.6 Statistical Analyses

Artemia DWT, total lipid per WWT, % FA composition, and FA ratios were compared among enrichments with analysis of variance (ANOVA) and post-hoc comparisons (Tukey's HSD). Larval DWT, stress test recovery time, total lipid per WWT, FA percent composition, and FA ratios were compared among enrichments and larval stages with ANOVA and Tukey's HSD. Survival and visual condition assessment of crabs from different diets were compared at each developmental stage using an ANOVA and Tukey's HSD. Fatty acid percentage data were log +1 transformed to meet the normality assumption of the model. To avoid Type 1 error in the ANOVA tests, p-values were Bonferroni corrected based on the number of lipid comparisons (n) per diet. All ANOVAs were run using the statistical program JMP, Version 11 (SAS Institute Inc., Cary, NC).

Principal component analysis (PCA) was used to simplify multivariate FA and lipid class data by transforming correlated variables into a set of uncorrelated principal components (Meglen, 1992). Eight highly variable lipid variables (16:1n-7, 18:1n-9, 20:5n-3, SumC20:1, SumC18, 22:6n-3, 16:0, 20:4n-6) were chosen to run the PCA based on biological significance for RKC larvae, including the main FA in our four diets. Proportions of these variables were used to run the PCA. All major trends could be accounted for with two principal components (PC1, PC2) without significant loss of the total original variation. PCA lipid loading coefficients are defined as the correlation coefficients between the original lipid variables and the PCA axis. PCA scores are defined as the position of the original variables along the new PCA axis (Meglen, 1992). The PCA was run using Minitab version 10.5 (Minitab Inc., State College, PA).

2.4 Results

2.4.1 Lipid Classes and Fatty Acids

Enrichment with four experimental emulsions produced *Artemia* with significantly different proportions (%) of FAs but similar overall lipid per WWT (Table 2.2). *Artemia* enriched with the DHA, DHA & EPA, and DHA & AA had lower levels of MUFA (25-29%), compared to *Artemia* receiving the control enrichment (40%, $F_{3,11}$=308.40, p<0.0001). *Artemia* enriched with the three HUFA treatments resulted in high levels of PUFA but variable proportions of DHA (9.3 – 16.9%), EPA (3.0 – 5.8%), and AA (0.5 – 7.1%). The control diet had a similar total lipid per WWT but had much lower proportions of DHA (0%), EPA (0.9%) and AA (0.3%, Table 2.2). Total lipids (µg/mg WWT) did not vary among the enrichments ($F_{3,11}$=1.54, p=0.28), and all *Artemia* contained 17-18% SFA (Table 2.2). The major lipid class in all the enrichment groups was TAG followed by PL, and percentages of TAG and PL did not vary significantly among *Artemia* enrichments (TAG: $F_{3,11}$=2.78, p=0.14; PL: $F_{3,11}$= 0.19, p=0.90; Table 2.3).

Changes in the percentage of SFA changed among the stages and among the diets at the G and C1 stages. Although the percentage of SFA varied; 16:0 remained the major SFA for all diets during all stages (Tables 2.4 – 2.6). During the Z4 stage, the total SFA was not different among all four diets ($F_{3,11}$=0.63, p=0.61); however, during the G stage crabs that received the DHA diet had higher levels of SFA (27.0%) than crabs that received the control (22.9%, $F_{3,23}$=5.59, p=0.006). At C1 SFA was greatest in crabs that received the DHA & EPA diet (26.0%) compared to those that received the other three diets (22-25%, $F_{3,12}$=17,1, p=0.0005).

During all stages, crabs that received the EFA diets had lower MUFA than crabs that received the control diet (Z4: $F_{3,11}$=130.33, p<0.0001, Table 2.4; G: $F_{3,23}$=129.29, p<0.0001, Table 2.5; C1: $F_{3,12}$=51.83, p<0.0001, Table 2.6). MUFA represented 25-31% of the total FA in crabs receiving the EFA diets, compared to 38-41% MUFA for the control diet. The primary

MUFA for all diets was 18:1n-9; and crabs receiving the EFA diets consisted of lower percentages of 18:1n-9 (Z4:18-19%, G:20-22%, C1:17-21%, Tables 2.4 – 2.6) than crabs receiving the control diet (Z4:29%, $F_{3,11}$=169.11, p<0.0001; G:31%, $F_{3,23}$=342.53, p<0.0001; C1:28%, $F_{3,12}$=76.77, p<0.0001). Crabs that received the EFA diets also had lower levels of 18:2n-6 ($F_{3,11}$=129.11, p<0.0001) and 18:1n-9 ($F_{3,11}$=318.90, p<0.0001) than those that received the control diet during all stages (Tables 2.4 – 2.6).

Polyunsaturated FAs in crabs ranged from 34-55%, and crabs that received the EFA diets had higher levels of PUFA during the Z4 (52-55%, $F_{3,11}$=55.79, p<0.0001, Table 2.4), G (41-43%, $F_{3,23}$=20.53, p<0.0001, Table 2.5), and C1 stages (~42%, $F_{3,12}$=7.55, p=0.008, Table 2.6) than crabs that received the control diet (Z4:44%, G:35%, C1:38%, Tables 2.4 – 2.6). Higher proportions of each EFA were found in larvae fed the respective enriched diet, with DHA highest for larvae receiving the DHA diet (Z4: $F_{3,11}$=882.14, p<0.0001; G: $F=_{3,23}$=417.00, p<0.0001), EPA highest in those receiving the DHA & EPA diet (Z4: $F_{3,11}$=520.02, p<0.0001; G: $F=_{3,23}$=251.43, p<0.0001), and AA highest for those receiving the DHA & AA diet (Z4: $F_{3,11}$=3244.79, p<0.0001; G: $F=_{3,23}$=1719.88, p<0.0001; Tables 2.4 – 2.6).

Table 2.2. Total fatty acids per WWT (µg/mg) and major fatty acids (>1.5%) in *Artemia* enriched with four experimental emulsions. Values are average ± SE (n=3).

	Control	DHA	DHA & EPA	DHA & AA
Total fatty acids (µg/mg WWT)	16.9 ± 2.2	19.2 ± 1.5	20.6 ± 0.6	17.1 ± 0.7
% total fatty acids				
16:0	11.1 ± 0.8	10.8 ± 0.5	10.6 ± 0.0	9.8 ± 0.1
18:0	4.8 ± 0.1	2.6 ± 1.3	4.1 ± 0.1	4.4 ± 0.2
ΣSFA[1]	17.1± 0.7	17.7 ± 0.6	17.8 ± 0.1	17.2 ± 0.2
16:1n7	1.4 ± 0.0	1.6 ± 0.0	3.2 ± 0.2	1.4 ± 0.0
18:1n9	34.0 ± 0.7	21.1 ± 0.3	19.6 ± 0.1	19.8 ± 0.1
18:17	4.5 ± 0.1	3.0 ± 0.2	4.0 ± 0.1	3.2 ± 0.1
C20:1	0.7 ± 0.0	0.5 ± 0.0	1.5 ± 0.0	0.6 ± 0.0
ΣMUFA[2]	40.7 ± 0.7	26.4 ± 0.4	29.3 ± 0.2	25.0 ± 0.2
18:2n6	8.4 ± 0.0	4.6 ± 0.2	4.8 ± 0.0	5.4 ± 0.1
18:3n3	23.6 ± 1.1	21.7 ± 1.0	22.7 ± 0.6	21.9 ± 0.7
18:4n3	4.4 ± 0.2	3.7 ± 0.2	4.3 ± 0.0	3.6 ± 0.1
20:3n3	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.1
20:4n6 (AA)	0.3 ± 0.0	0.2 ± 0.1	0.5 ± 0.0	7.1 ± 0.3
20:5n3 (EPA)	0.9 ± 0.1	3.6 ± 0.1	5.8 ± 0.1	3.0 ± 0.0
22:6n3 (DHA)	0 ± 0.0	17.0 ± 1.6	9.3 ± 0.6	11.5 ± 1.0
ΣPUFA[3]	40.4 ± 1.3	54.3 ± 0.9	51.0 ± 0.1	56.1 ± 0.4
DHA/EPA	0 ± 0.0	4.8 ± 0.6	1.6 ± 0.1	3.8 ± 0.4

[1] Includes 14:0, i15:0, ai15:0, i17:0, ai17:0, 17:0, 15:0, 20:0, 22:0
[2] Includes 14:1, 16:1n5, 20:1n11, 22:1n11
[3] Includes 16:2n4, 16:3n4, 16:4n3, 18:2n4, 20:2n6, 20:3n6, 22:5n3

Table 2.3. Lipid class composition (% total lipids) of *Artemia* enriched for 24 h using four different oil emulsions (average ± SE, n=3).

	Control	DHA	DHA & EPA	DHA & AA
Lipid Class (% total lipid)				
Triacylglycerols	43.5 ± 7.0	35.7 ± 11.2	46.2 ± 5.5	43.7 ± 4.1
Free fatty acids	27.1 ± 5.3	26.1 ± 3.2	22.6 ± 5.2	20.6 ± 2.5
Sterols	3.4 ± 1.4	11.0 ± 6.1	4.3 ± 0.3	4.2± 0.2
Polar lipids	26.0 ± 1.2	26.9 ± 2.7	26.8 ± 0.6	31.5 ± 4.1

The total amount of FA per WWT increased during the zoeal stages and then decreased during the nonfeeding glaucothoe and juvenile stages (Tables 2.4 – 2.6). The amount of total FA was not significantly different among the diets during Z4 ($F_{3,11}$=0.94, p=0.97) and C1 stages ($F_{3,11}$=1.74, p=0.24); however, there was a significant difference during the G stage ($F_{3,23}$=4.74, p=0.01), with crabs receiving the DHA diet (24.5 ± 3.0 µg/mg WWT) having more total FA than crabs that received the control diet (12.5 ± 1.9 µg/mg WWT). The same increasing then decreasing trend was found for the average total lipid per WWT for all four diets, with an increase from 72 to 136 µg/mg WWT from Z1 to Z4 and decrease from 90 to 49 µg/mg WWT from G to C1 (Figure 2.1). Total lipids consisted of different percentages of DHA (Figure 2.2a), EPA (Figure 2.2b), and AA (Figure 2.2c) among the diets. Ratios of DHA:EPA (Figure 2.3a) and EPA:AA (Figure 2.3b) also varied among the diets at different stages. During the Z4 stage, crabs receiving the DHA and the DHA & EPA diet had higher ratios of DHA:EPA than crabs receiving the other two diets ($F_{3,11}$=3469.93, p<0.0001). Crabs receiving the DHA and the DHA & EPA diet had a higher ratio of EPA:AA than the other two diets ($F_{3,11}$=1377.24, p<0.0001). Ratios followed the same pattern during the G stage. At the C1 stage, the ratio of DHA:EPA did not vary among larvae receiving EFA-enriched diets and was in the range 1 to 1.5, whereas crabs that received the control diet had a significantly lower ratio of 0.5 ($F_{3,12}$=19.69, p=0.0003).

Table 2.4. Total fatty acids per WWT (µg/mg) and major fatty acids (>1.5%) in Z1 and Z4 RKC larvae fed four different diets. Values are average ± SE (n=3).

		Control	DHA	DHA & EPA	DHA & AA
	Z1	Z4	Z4	Z4	Z4
Total fatty acids (µg/mg WWT)	15.7 ± 2.2	31.7 ± 1.9	26.8 ± 2.5	31.7 ± 3.7	28.2 ± 1.7
% total fatty acids					
16:0	15.6 ± 0.5	9.5 ± 0.1	12.4 ± 0.4	11.4 ± 0.3	11.3 ± 0.3
18:0	5.5 ± 0.3	4.9 ± 0.1	5.0 ± 0.1	5.1 ± 0.0	5.6 ± 0.1
20:0	1.5 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
ΣSFA[1]	25.2 ± 1.1	17.3 ± 2.0	19.2 ± 0.5	18.1 ± 0.3	18.4 ± 0.3
16:1n7	3.4 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.8 ± 0.0	0.9 ± 0.0
18:1n9	14.1 ± 0.2	29.4 ± 0.8	19.4 ± 0.2	18.8 ± 0.1	18.1 ± 0.0
18:1n7	12.2 ± 0.1	6.3 ± 0.1	5.0 ± 0.0	6.3 ± 0.0	5.0 ± 0.0
C20:1	4.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.9 ± 0.0	1.3 ± 0.0
ΣMUFA[2]	35.2 ± 0.4	38.0 ± 1.0	26.8 ± 0.2	29.1 ± 0.2	25.3 ± 0.1
18:2n6	0.8 ± 0.0	8.1 ± 0.2	4.5 ± 0.0	4.8 ± 0.0	5.0 ± 0.0
18:3n3	0.2 ± 0.0	23.4 ± 0.6	18.0 ± 0.3	19.2 ± 0.1	16.6 ± 0.2
18:4n3	0.2 ± 0.0	2.5 ± 0.0	1.6 ± 0.0	1.2 ± 0.6	1.3 ± 0.0
20:4n6 (AA)	4.6 ± 0.1	1.0 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	8.4 ± 0.1
20:3n-3	0.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.1	2.3 ± 0.0	2.5 ± 0.1
20:5n3 (EPA)	18.5 ± 0.7	3.2 ±0.1	6.9 ± 0.1	8.9 ± 0.2	5.4 ± 0.0
22:6n3 (DHA)	9.6 ± 0.4	1.3 ± 0.0	17.0 ± 0.2	11.6 ± 0.4	13.3 ± 0.4
ΣPUFA[3]	38.5 ± 1.6	43.7 ± 1.0	52.9 ± 0.7	51.7 ± 0.4	55.2 ± 0.4
DHA/EPA	0.5 ± 0.0	0.4 ± 0.0	2.5 ± 0.0	1.3 ± 0.0	2.5 ± 0.0

[1] Includes 14:0, 17:0, i15:0, ai15:0, i16:0, 15:0, i17:0, ai17:0, 22:0
[2] Includes 14:1, 16:1n5, 18:1n11, 18:1n6, 18:1n5, 20:1n9, 20:1n7
[3] Includes 16:2n4, 16:3n4, 16:4n3, 18:2n4, 20:2n6, 20:3n6, 21:5n3, 22:4n6, 22:5n6, 22:5n3

Table 2.5. Total fatty acids per WWT (µg/mg) and major fatty acids (>1.5%) in RKC G fed four different diets. Values are average ± SE (n=6).

	Control G	DHA G	DHA & EPA G	DHA & AA G
Total fatty acids (µg/mg WWT)	12.5 ± 1.9	24.5 ± 3.0	19.3 ± 2.2	18.9 ± 1.9
% total fatty acids				
16:0	11.0 ± 0.4	14.2 ± 0.3	12.7 ± 0.1	13.2 ± 0.1
18:0	9.6 ± 0.6	8.1 ± 0.4	7.7 ± 0.2	8.4 ± 0.2
20:0	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1
22:0	0.2 ± 0.1	2.2 ± 0.2	1.4 ± 0.1	1.5 ± 0.1
ΣSFA[1]	22.9 ± 1.0	27.4 ± 1.1	24.5 ± 0.6	26.0 ± 0.5
16:1n7	1.2 ± 0.0	1.0 ± 0.0	1.7 ± 0.0	0.9 ± 0.0
18:1n9	31.6 ± 0.5	21.8 ± 0.1	20.4 ± 0.2	20.0 ± 0.2
18:1n7	6.3 ± 1.3	6.2 ± 0.1	7.4 ± 0.1	6.1 ± 0 .0
C20:1	1.4 ± 0.1	1.9 ± 0.0	2.5 ± 0.1	2.2 ± 0.0
ΣMUFA[2]	41.4 ± 0.8	31.3 ± 0.1	32.7 ± 0.3	29.7 ± 0.2
18:2n6	7.6 ± 0.0	4.7 ± 0.1	5.1 ± 0.1	5.3 ± 0.0
18:3n3	18.6 ± 0.1	15.8 ± 0.5	16.8 ± 0.3	15.2 ± 0.1
18:4n3	1.2 ± 0.4	1.3 ± 0.1	1.5 ± 0.1	0.8 ± 0.3
20:4n6 (AA)	0.8 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	6.4 ± 0.1
20:3n-3	1.4 ± 0.0	1.8 ± 0.1	1.8 ± 0.0	1.9 ± 0.0
20:5n3 (EPA)	2.4 ± 0.0	4.8 ± 0.1	6.4 ± 0.2	3.8 ± 0.1
22:6n3 (DHA)	0.7 ± 0.1	9.2 ± 0.4	6.6 ± 0.2	7.0 ± 0.2
ΣPUFA[3]	34.7 ± 0.4	40.0 ± 1.2	41.4 ± 0.8	43.1 ± 0.6
DHA/EPA	0.3 ± 0.0	1.9 ± 0.0	1.0 ± 0.0	1.8 ± 0.0

[1] Includes 14:0, i15:0, 15:0, i17:0, ai17:0, 17:0
[2] Includes 14:1, 16:1n9, 16:1n5, 18:1n6, 18:1n5, 20:1n11
[3] Includes 16:2n4, 16:3n4, 16:4n3, 18:2n4, 20:2n6, 20:3n6, 22:5n3

Table 2.6. Total fatty acids per WWT (µg/mg) and major fatty acids (>1.5%) in C1 RKC fed four different diets. Values are average ± SE (n=3).

	Control C1	DHA C1	DHA & EPA C1	DHA & AA C1
Total fatty acids (µg/mg WWT)	13.3 ± 1.3	10.4 ± 0.3	10.0 ± 1.5	10.1 ± 1.4
Percentage total fatty acids				
16:0	12.2 ± 0.1	15.3 ± 0.8	14.8 ± 0.1	16.5 ± 0.7
18:0	8.3 ± 0.2	7.2 ± 0.3	7.4 ± 0.0	9.1 ± 0.3
20:0	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.8 ± 0.2
ΣSFA[1]	22.4 ± 0.1	24.9 ± 1.2	24.5 ± 0.1	28.8 ± 1.0
16:1n7	1.0 ± 0.0	1.1 ± 0.1	1.3 ± 0.1	0.9 ± 0.0
18:1n9	28.1 ± 0.5	20.7 ± 0.3	19.0 ± 0.3	17.7 ± 0.9
18:1n7	8.1 ± 0.1	7.4 ± 0.4	8.3 ± 0.0	7.1 ± 0.0
18:2n6	7.3 ± 0.1	3.2 ± 0.4	2.8 ± 0.1	1.9 ± 0.2
C20:1	1.2 ± 0.0	1.4 ± 0.1	1.9 ± 0.1	1.6 ± 0.1
ΣMUFA[2]	39.1 ± 0.5	31.6 ± 0.9	31.5 ± 0.3	28.0 ± 0.9
18:3n-3	15.2 ± 0.3	10.9 ± 0.4	11.0 ± 0.5	8.6 ± 0.6
20:4n6 (AA)	1.7 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	9.1 ± 0.3
20:3n3	1.6 ± 0.0	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.1
20:5n3 (EPA)	5.6 ± 0.2	8.4 ± 0.2	9.8 ± 0.5	6.4 ± 0.4
22:6n3 (DHA)	2.0 ± 0.1	11.9 ± 1.1	10.7 ± 0.5	9.0 ± 2.0
ΣPUFA[3]	37.6 ± 0.5	42.5 ± 0.3	42.9 ± 0.3	42.3 ± 1.8
DHA/EPA	0.4 ± 0.0	1.4 ± 0.1	1.1 ± 0.0	1.4 ± 0.3

[1] Includes 14:0, i15:0, i16:0, ai16:0, i17:0, ai17:0, 17:0, 15:0, 22:0
[2] Includes 14:1, 16:1n9, 16:1n5, 18:1n6, 18:1n5,
[3] Includes 16:2n4, 16:3n4, 16:4n3m 18:2n4, 18:4n3, 20:2n6, 20:3n6, 22:4n6

The first two axes of the principal component analyses (PCA) described 70% of the variance in the lipid composition of *Artemia* and RKC from all four dietary treatments (Figure 2.4). The first principal component (PC 1) explained most of the variation (53%) and displayed a separation of long chain PUFAs, including DHA, EPA and AA (on positive "PUFA" side of the

axis), from shorter C_{18} FAs (on negative MUFA side of the axis) (Figure 2.4a). PC 2 explained less variance (17%) and separated 20:4n-6 from other PUFA. Each of the enrichments grouped together on the PCA with some separation among larval stages and between larval stages and *Artemia* (Figure 2.4b). Larvae fed the EFA enrichments were further on the positive side of PC 1, as a function of their higher levels of PUFA. Conversely, larvae fed the control diet were on the negative side of PC 1, indicating lower levels of PUFA and higher levels of 18:1n-9. The later developmental stages of larvae shifted to the more positive end of PC 1. Larvae and *Artemia* from the DHA+AA diet separated along PC 2, reflecting the high input of AA into their diets.

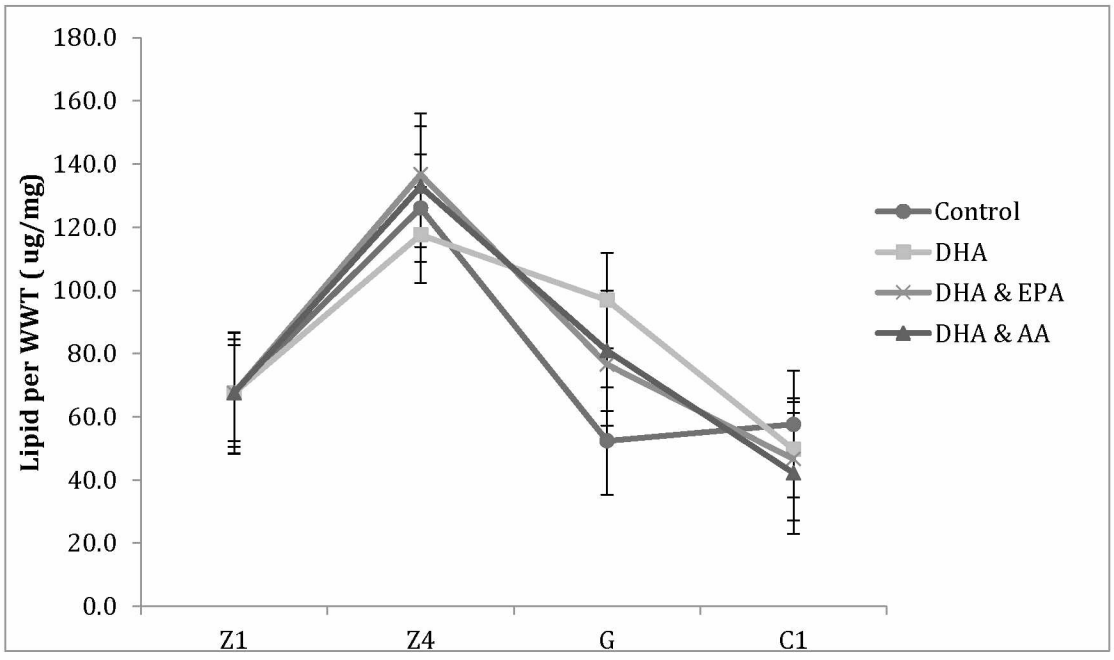

Figure 2.1. Average (± SE) lipid per WWT (µg/mg) for crabs fed enriched diets (Z1 and Z4 n=30, G n = 12, C1 n = 36.)

2.4.2 Growth

King crab larvae grew substantially during the experiment, increasing from an average of 0.6 ± 0.01 µg WWT per individual at the Z1 stage to an average of 3 ± 0.12 µg WWT per individual at the Z4 stage. Larval WWT, DWT, and AWT did not vary among diets at the Z1, Z4, and C1 stages. Larval WWT varied significantly at the G stage, when larvae fed the DHA & EPA enriched diets were larger (3.7 ± 0.10 µg WWT per individual) than those that received the DHA diet, the DHA & AA diet, and the control diet (2.9 ± 0.24, 3.0 ± 0.09, 3.1 ± 0.32 µg WWT per individual respectively; $F_{3,23}$=4.58, p=0.01). Larval DWT for crabs that received the DHA & EPA diet (0.79 ± 0.01 µg DWT per individual) were also significantly different during the G stage compared to crabs fed the DHA, DHA & AA, and the control diets (0.65 ± 0.05, 0.64 ± 0.02, 0.61 ± 0.03 µg DWT per individual respectively; $F_{3,23}$=7.42, p=0.002). Larval AWT did not vary significantly among the diets during any of the stages.

2.4.3 Survival and Visual Condition Assessment

Average survival across all treatments from Z1 to Z4 was 78 ± 0.06 % and dropped to an average of 21 ± 0.07% at the C1 stage (Figure 2.5). Survival among the four diets was not significantly different overall ($F_{3,71}$=1.67, p=0.1856) or by stage (diet*stage interaction, $F_{15,71}$=1.04, p=0.43); however, survival decreased with progressive stages ($F_{5,71}$=131.60, p<0.0001).

The visual condition assessment of glaucothoe from different dietary groups showed significant differences in the number of lipid droplets ($F_{3,239}$=15.79, p<0.0001) and the maximum lipid droplet diameter ($F_{3,239}$=18.90, p<0.0001; Figures 2.6 and 2.7). The highest numbers of lipid droplets were counted in glaucothoe from the DHA & EPA (average 10 ± 0.4 droplets) and the DHA & AA (average 9.5 ± 0.1) dietary treatments. Glaucothoe that received the DHA and

control diets had significantly fewer lipid droplets averaging 7.5 ± 1.0 and 7.4 ± 0.5, respectively. The largest lipid droplets were found in crabs that received the DHA & EPA diet (126 μm) with crabs fed the control diet having the smallest droplets (102 μm). There were no significant differences between tanks within the same dietary treatment for either the number of lipid droplets ($F_{8,239}$= 0.91, p=0.50) or maximum lipid droplet diameter ($F_{8,239}$= 1.27, p=0.26).

2.4.4 Stress Test

Glaucothoe that received EFA-enriched diets recovered significantly faster from the salinity stress challenge (average ± SE, 384 ± 35 seconds) than those that had received the control diet (1169 ± 21 seconds, $F_{3,119}$=33.68, p<0.0001; Figure 2.8). There was no significant difference in FAs between the fast recovery and slow recovering crabs ($F_{1,16}$=4.11, p=0.06). The total amount of FAs per WWT (mg/g) and percentages of individual FAs did not differ between crabs that recovered quickly or slowly during the stress test ($F_{1,16}$=0.13, p=0.73). The DWT per individual did not vary between the quick and slowly recovering crabs for any of the diets.

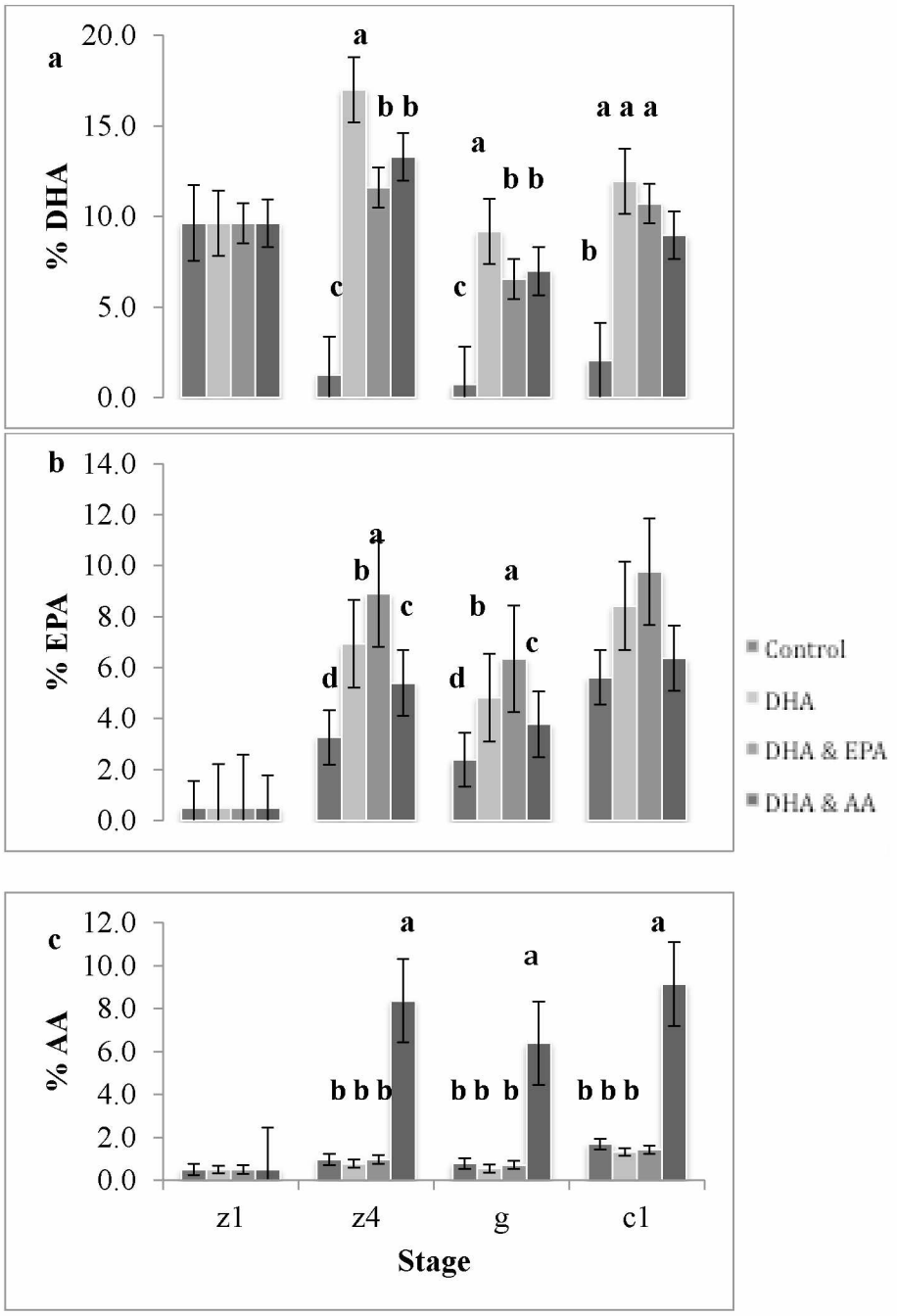

Figure 2.2. Relative proportions of (a) DHA, (b) EPA, and (c) AA for each diet by stage. Values are average ± SE (Z1 and Z4 n-3; G n=6; C1 n = 3). Results of a one-way ANOVA with Tukey's multiple comparison test are shown with letters representing a significant difference among dietary groups by stage.

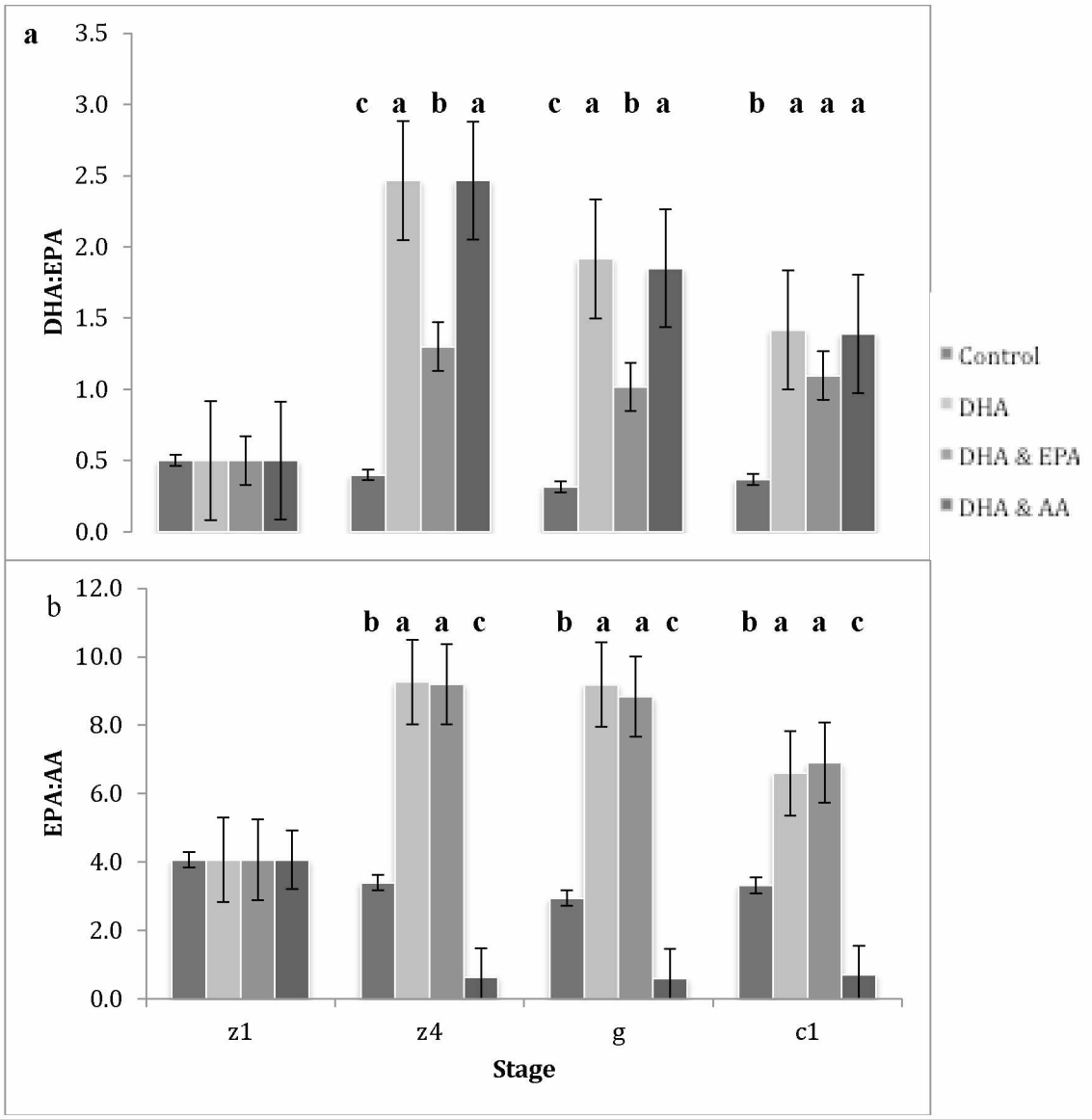

Figure 2.3. Relative proportions of (a) DHA:EPA and (b) EPA:AA for diet by stage.
Data represent the average ± SE (Z1, Z4, and C1 n = 3; G n = 6). Different letters represent a significant difference among dietary groups by stage; one-way ANOVA with Tukey's multiple comparison test.

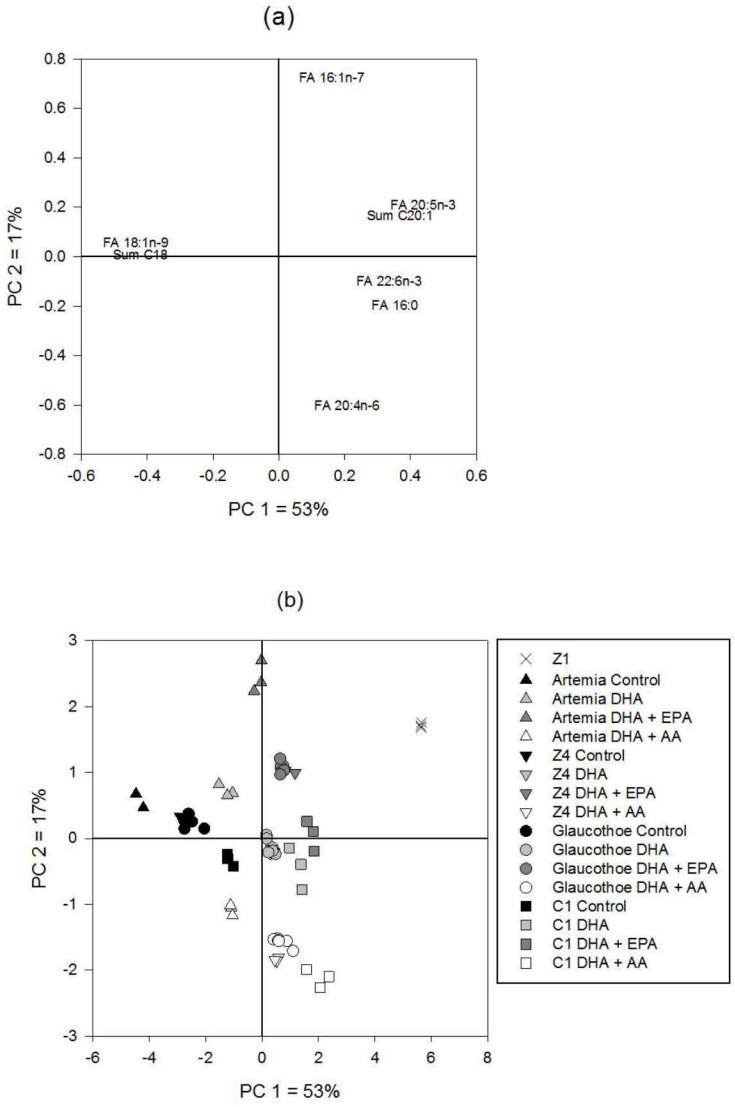

Figure 2.4. Principal component analysis for *Artemia* and crabs fed four different diets. (a) Lipid parameter coefficients and (b) RKC and *Artemia* scores for the first two principal components. The FA and lipid class parameters used were: 16:1n-7, 20:5n-3, C20:1, 22:6n-3, 16:0, 20:4n-6, Sum C18, and 18:1n-9.

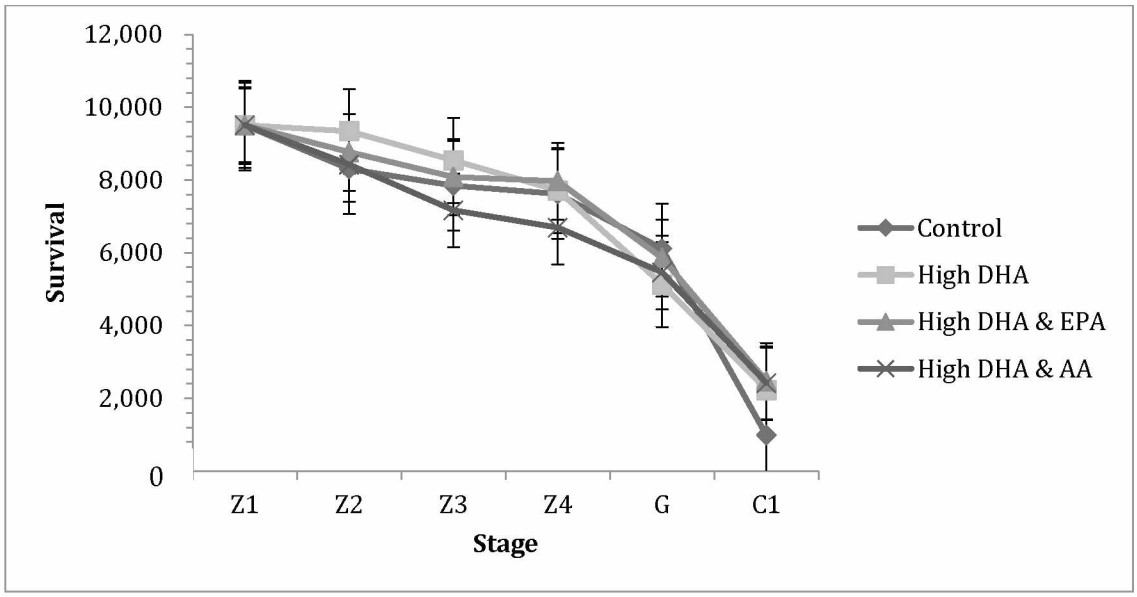

Figure 2.5. Average (± SE) survival of larvae and juveniles for each diet treatment by stage (n=3). Each tank initially contained 9,500 Z1 larvae. Survival among the four diets did not vary significantly for any of the larval stages. Survival averaged 59% from the Z1 to the G stage and then dropped to 36% from the G to C1 stage.

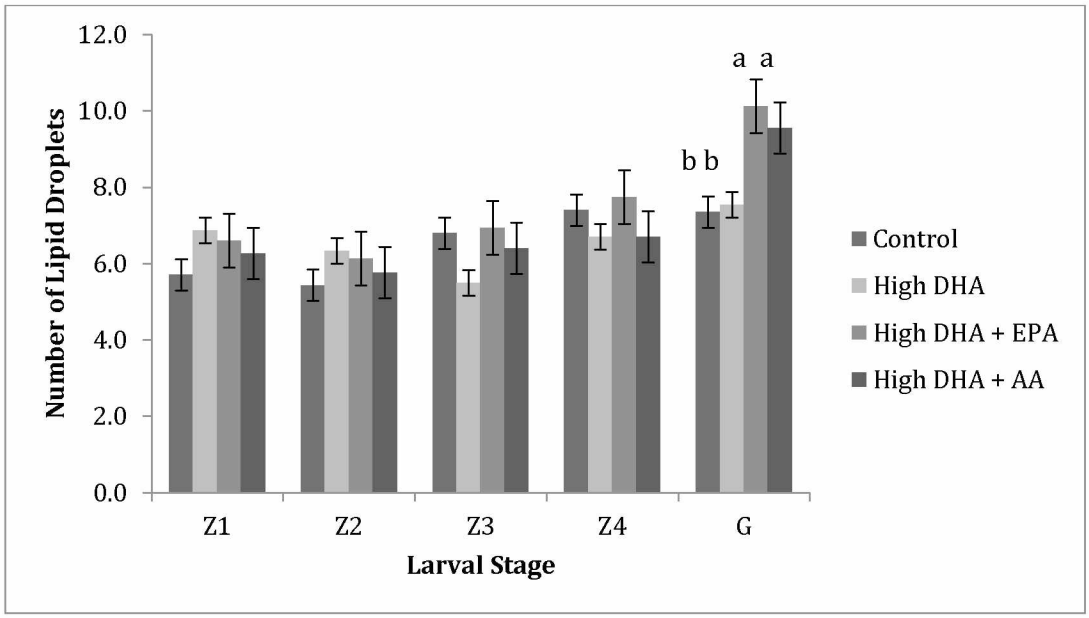

Figure 2.6. Average (± SE) number of lipid droplets for each diet by ontogenetic stage (G: n=30, $p<0.01$). Different letters represent a significant difference in the number of lipid droplets among the diets during the glaucothoe stage ($F_{3,239}=15.79$, $p<0.0001$).

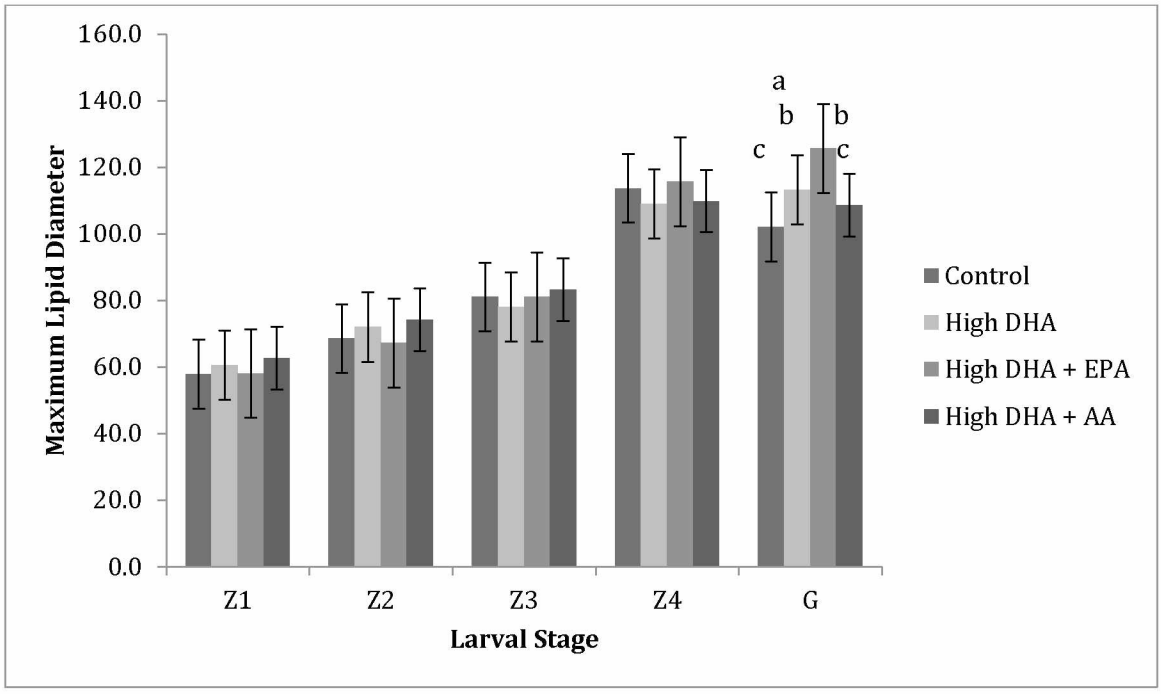

Figure 2.7. Average (± SE) maximum lipid diameter (µm) for all diets from the Z1 to G stage (G: n=30, $p<0.01$). Different letters represent a significant difference in lipid diameter among the diets during the glaucothoe stage ($F_{3,239}=18.90$, $p<0.0001$).

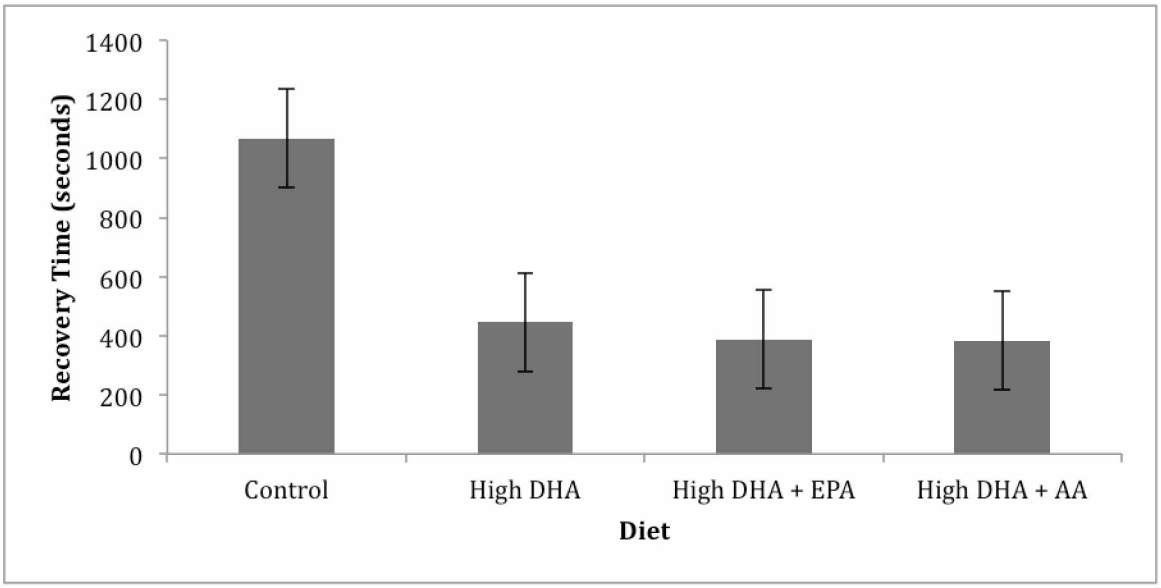

Figure 2.8. Average (± SE) recovery time (seconds) by diet for fresh water stress test (n=30). Crabs who received the control diet took a significantly longer time to recover ($F_{3,119}=33.68$, $p<0.0001$) than those fed the PUFA diets.

2.5 Discussion

This is the first study to examine the effect of dietary lipid on the growth, survival, and stress resistance of RKC during their early ontogeny. Diets were chosen to provide information on the importance of DHA, EPA, and AA for RKC larvae with total FAs higher for all diets compared to that found in the *Artemia*. Diets containing DHA, EPA, and AA improved the condition and the ability of larvae to handle stress. Although survival was not impacted, this study showed the importance of EFAs in RKC diets.

Differences between dietary FA ratios were quickly evident in early larval stages and were detectable in successive developmental stages of crabs. Total FAs were highest in the zoeal stages and became depleted in later development stages for all four diets. The composition of the FAs changed as well. The total PUFA percentage was in the fiftieth percentile in Z4 fed the EFA diets, which decreased into the fortieth percentiles during the G and C1 stages. Diets used in this study were providing adequate energy as shown by the storage of FAs. Decreases in these stores during the non-feeding G stage furthermore show their importance for survival during this critical period. Similar patterns have been seen in blue king crab larvae raised in hatcheries (Copeman et al., 2014).

Comparing lipid content among species may give a better understanding of the lipid demand required for animals living in different environments. Stone crabs raised in a hatchery had lower total lipids at the Z1 (stone crab: 2.5 ± 0.4 % DWT; RKC: 77.3 ± 4.4 % DWT), Z4 (stone crab: 25.3 ± 3.0 % DWT; RKC: 53.6 ± 5.7 % DWT), and megalope (stone crab: 16.4 ± 7.4 % DWT; RKC: 66.4 ± 6.0 % DWT) stages (Nates and McKenney, 2000) compared to crabs in this study. Studies have also found lower total lipids (% DWT) in Chinese mitten crabs when raised in hatcheries at the Z1 (5.9) and megalope stages (11.28-17.64; Sui et al., 2007). Copeman

et al. (2014) found that blue king crab raised on a DHA Selco diet had more total lipids at the Z4 stage (77.2 ± 1.8 µg/mg WWT) and early G stage (67.9 ± 2.9 µg/mg WWT) but less total lipids at the C1 stage (29.6 ± 4.4) then crabs in this study (Tables 2.4-2.6). The RKC from all four diets in this study contained a higher total lipid content than other crab species such as Artic lyre crabs (3.2 µg/mg lipid WWT) and hermit crabs (14.3 ug/mg lipid WWT; Copeman and Parrish, 2003). These differences in total lipids may result from factors such as diet, water temperature, or requirements for different species. A future study could investigate changes in total lipids of hatchery raised RKC larvae at different temperatures along with a variety of diets to determine the interaction of temperature and diet.

This experiment showed that RKC larvae are not sensitive to dietary HUFA ratios but do require high levels of HUFA in their diets. HUFAs are not only important for RKC but have proved important for other species, such as spiny rock lobsters, mud crab, and Chinese mitten crab (Conlan et al., 2014; Sheen and Wu, 2002; Sui et al., 2007; Suprayudi et al., 2004a). Successful survival of spiny rock lobster (*Panulirus ornatus*) larvae requires food sources rich in AA, EPA, and DHA (Conlan et al., 2014). The same has been found for mud and Chinese mitten crabs (Sheen and Wu, 2002; Sui et al., 2007; Suprayudi et al., 2004a). Including EFAs in diets also increases weight for mud crab (Sheen and Wu, 2002) and allows for larger carapaces and bigger megalopae for Chinese mitten crabs (Sui et al., 2007; Suprayudi et al., 2004a). Our study found that the percentages of HUFA increased from the Z1 to the Z4 stage with levels of DHA, EPA, and AA continuing to increase into the G and C1 stages. The relative conservation of these FAs during this non-feeding stage shows the importance of these FAs for king crab larvae.

Evaluating the number of lipid droplets and their size can provide the general condition of each larval tank. This visual health index (i.e number and size of droplets) is complementary

to the biochemical analyses. The two diets that resulted in increased visual lipid during the glaucothoe stage were the DHA & EPA and DHA & AA diets. The glaucothoe stage may be the best time to observe larval condition because crabs are going through major morphological changes along with utilizing energy reserves. Looking at the lipid composition of larvae at this stage, specifically TAGs, can also provide an insight as to their condition. Triacylglycerols were the largest lipid class in our enrichments (35-46%) followed by PL (26-31%). Triacylglycerols are considered an energy storage that can often be seen as oily droplets (Hakanson, 1984). Highest absolute amounts of TAG were found in the DHA & EPA diet, which may explain why glaucothoe that received this diet had larger and more lipid droplets. Amounts of TAG can help assess condition since it is correlated with increases in survival and metamorphosis success (Fraser, 1989; Ouellet et al., 1992). Growth and molting success for Chinese mitten crabs (*Eriocheir sinensis*) has been related to the storage of TAGs (Wen et al., 2006). During larval development, TAG accumulates in American lobsters (Sasaki, 1984; Sasaki et al., 1986) and is an important energy store that is catabolized during starvation (Sasaki, 1984). Quantification of lipids has been done in a number of species and is useful in estimating the condition of larvae (Angel-Dapa et al., 2010; Gallager and Mann, 1986a; Gallager et al., 1986; Rodriguez-Jaramillo et al., 2008). Lipid droplets stored in the digestive gland have been assessed for size and number in prawns (*Macrobrachium amazonicum,* (Anger and Hayd, 2009). As prawns were starved the number of droplets was found to decrease. Survival of hard clams (*Mercenaria mercenaria*) and Eastern oysters (*Crassostrea virginica*) is also correlated to the number of lipid droplets (Gallager and Mann, 1986b). Survival of eggs to straight hinge and pediveliger stage in hard clams and Eastern oysters is correlated with the number of lipid droplets in eggs. Having a quick,

reliable condition assessment is important for successful culturing and the release of healthy individuals.

Inclusion of dietary HUFA allowed better survival of salinity stress tests in RKC non-feeding glaucothoe. The effect of diet on stress response has been observed in Chinese Mitten crabs, blue shrimp (*Penaeus stylirostris*) and Carpus shrimp (*Farfantepenaeus paulensis*; Chim et al., 2001; Martins et al., 2006; Sui et al., 2007). Chinese mitten crabs tolerance to salinity increased as higher levels of n-3 HUFAs, including DHA and EPA, were included in the diet (Sui et al., 2007). Diets containing as high as 50% total n-3 HUFA increase tolerance to salinity by allowing the Chinese mitten crabs to survive longer in higher salinity waters. Higher HUFA diets also help blue shrimp with regulation of osmolality increasing survival when encountering salinity changes (Chim et al., 2001). Lower mortality has also been found for Carpus shrimp when fed a diet consisting of 50% n-3 HUFA compared to unenriched *Artemia* and SFA diets (Martins et al., 2006) showing the importance of HUFA in their diet.

Similar survival among diet treatments indicates that FA ratios in our diets did not overcome the survival bottleneck at the glaucothoe stage. In comparison to prior studies, we observed excellent survival with 59% of the larvae surviving to the G stage and 21% to the C1 stage compared to 47-53% to the G stage and 20% to the C1 stage in Alaska (Swingle et al., 2013) and 35% to the G stage in Russia (Borisov et al., 2007). The lack of difference in survival among diet treatments was surprising, since EFAs have been found to improve survival for other crustaceans. Chinese mitten crab megalopae experience higher survival (ranging from 61.92 ± 6.01 to 67.50 ± 3.79%) when receiving higher ratios of n-3 FAs then when continuously fed lower enrichment diets (52.33 ± 6.15%; Sui et al., 2007). Diets with DHA:EPA ratios of 2 and 4 have proved beneficial in increasing survival and reducing metamorphosis failure for other crab

species (Nghia et al., 2007; Sui et al., 2007). Our diets did not provide such ratios of DHA:EPA so increasing the ratio of DHA:EPA in our diets might increase the survival of king crab larvae. Investigating the impacts of diets with just EPA and AA on crab survival and condition may reveal their role in the absence of DHA. Differences in ratio requirements between stocks have been found for other species (Nghia et al., 2007), and conducting this same diet study on other Alaska RKC stocks could determine if required ratios of DHA:EPA:AA change and whether one diet would be adequate for raising larvae from different stocks around the state.

The proportions of EFAs in the emulsions used to enrich *Artemia* in this study were chosen based on levels found in wild RKCs, including a DHA:EPA ratio of 0.6 (Copeman et al., 2012). Larvae that received the DHA diet had similar percentages of DHA to those found in wild juveniles (Table 2.7). Ratios of DHA:EPA for all juveniles in this study were higher than that found in wild king crab. Wild RKC C2s had fewer total FA but higher percentages of PUFA, including DHA, EPA, and AA compared to hatchery-raised C2s fed a variety of commercially available foods along with DHA Selco enriched *Artemia* (Table 2.7); whereas juvenile crabs fed the DHA, DHA & EPA, and the control diet in this study had lower percentages of these FA compared to crabs raised on a DHA Selco diet (Copeman et al., 2012). Juveniles that received the DHA & AA diet also had lower percentages of DHA & EPA compared to crabs fed a DHA Selco diet but had a higher percentage of AA. Total FAs were higher in juveniles that received a DHA Selco (13.0 ± 1.5 µg/mg WWT) diet than crabs fed the EFA diets in this study. Research on the FA composition of all wild larval stages is needed in order to determine the optimal ratios of FA during each larval stage. Samples of wild C1 may help to better define the appropriate ratios of these EFAs. Since the G stage continues to be a bottleneck, comparisons between wild and hatchery glaucothoe may be most informative in determining what ratios of these EFAs are

needed at the beginning of this non-feeding period to allow the crabs to make it through this critical life stage.

Table 2 7. Fatty acid composition of wild RKC and cultured RKC juveniles fed a diet of DHA Selco from (Copeman et al., 2012)

Diet	Total FA (µg/mg WWT)	% PUFA	% DHA	% EPA	% AA
Wild RKC	8.6 ± 1.5	49.0 ± 2.5	14.4 ± 1.4	24.7 ± 1.3	2.8 ± 0.3
Cultured RKC on DHA Selco	14.4 ± 1.3	43.1 ± 1.4	13.0 ± 0.4	13.3 ± 0.5	1.8 ± 0.1

Effective hatchery methods are important to ensure successful culturing. Commercial enrichments, such as DHA Selco, are used in hatcheries and have been the primary enrichment used to raise Alaska king crab (Swingle et al., 2013). This enrichment process is similar to that provided for other cultured species, however some hatcheries add emulsions every 12 hours (Lavens and Sorgeloos, 1996) unlike the 24 hours used in this experiment. DHA Selco provides a DHA:EPA ratio of 1.6 µg/mg DWT (Lavens and Sorgeloos, 1996), which is similar to what was provided in this experiment. The enrichment process in this study required additional steps during enrichment compared to that used when enriching with DHA Selco. Including an emulsifier similar to that used in DHA Selco should be considered for future emulsion experiments to simplify methods.

Soon after *Artemia* were first used in aquaculture, their nutritional deficiencies were recognized and enrichments were created, with fish and shrimp larvae in mind (Sorgeloos et al., 2001). Red king crab larvae responded positively to EFA diet enrichments with improved larval condition and performance compared to larvae fed the control diet documented in this study. The hatchery bottleneck during the glaucothoe stage was not surpassed with the enrichments used here or those found in DHA Selco (Swingle et al., 2013), therefore, further studies are needed to investigate potential supplements or solutions to improve survival during this challenging stage in hatchery culture. More work needs to be conducted using different ratios of DHA, EPA, and AA to see if survival can be increased. Further investigations should also focus on the ratios of EFAs in wild glaucothoe compared to hatchery animals to develop a diet that may increase survival during this critical stage.

2.6 References

Abrunhosa, F.A., Kittaka, J., 1997a. Morphological changes in the midgut, midgut gland, and hindgut during the larval and postlarval development of the red king crab *Paralithodes camtschaticus*. Fish. Sci. 63, 746-754.

Abrunhosa, F.A., Kittaka, J., 1997b. Functional morphology of mouthparts and foregut of the last zoea, glaucothoe and first juvenile of the king crabs *Paralithodes camtschaticus*, *P. brevipes* and *P. platypus*. Fish. Sci. 63, 923-930.

Agnalt, A.L., 2008. Fecundity of the European lobster (*Homarus gammarus*) off southwestern Norway after stock enhancement: do cultured females produce as many eggs as wild females? ICES J. Mar. Sci. 65, 164-170.

Andres, M., Estevez, A., Hontoria, F., Rotllant, G., 2010. Differential utilization of biochemical components during larval development of the spider crab *Maja brachydactyla* (Decapoda: Majidae). Mar. Biol. 157, 2329-2340.

Angel-Dapa, M.A., Rodriguez-Jaramillo, C., Caceres-Martinez, C.J., Saucedo, P.E., 2010. Changes in lipid content of oocytes of the penshell *Atrina maura* as a criterion of gamete development and quality: a study of histochemistry and digital image analysis. J. Shellfish Res. 29, 407-413.

Anger, K., Hayd, L., 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquat. Bio. 7, 19-30.

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58, 122-137.

Bannister, R.C.A., Addison, J.T., 1998. Enhancing lobster stocks: a review of recent European methods, results, and future prospects. Bull. Mar. Sci. 62, 369-387.

Barclay, W., Zeller, S., 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia* nauplii by feeding spray-dried *Schizochytrium* sp. J. World Aquacult. Soc. 27, 314-322.

Berge, J.P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In: Gal, Y.L., Ulber, R. (Eds.), Marine Biotechnology I, pp. 49-125.

Borisov, R.R., Epelbaum, A.B., Kryakhova, N.V., Tertitskaya, A.G., Kovatcheva, N.P., 2007. Cannibalistic behavior in red king crabs reared under artificial conditions. Russian Journal of Marine Biology. 33, 227-231.

Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar. Mamm. Sci. 22, 759-801.

Champe, P.C., Harvey, R.A., Ferrier, D.R., 2008. Biochemistry, 4th ed. Wolters Kluwer Health/Lippincott Williams and Wilkins, Philadelphia, PA.

Chim, L., Lemaire, P., Delaporte, M., Le Moullac, G., Galois, R., Martin, J., 2001. Could a diet enriched with n- 3 highly unsaturated fatty acids be considered a promising way to enhance the immune defences and the resistance of penaeid prawns to environmental stress? Aquacult. Res. 32, 91-94.

Conlan, J.A., Jones, P.L., Turchini, G.M., Hall, M.R., Francis, D.S., 2014. Changes in the nutritional composition of captive early-mid stage *Panulirus ornatus* phyllosoma over ecdysis and larval development. Aquaculture. 434, 159-170.

Copeman, L., Daly, B., Eckert, G.L., Swingle, J., 2014. Storage and utilization of lipid classes and fatty acids during the early ontogeny of blue king crab, Paralithodes platypus. Aquaculture. 424, 86-94.

Copeman, L.A., Parrish, C.C., 2003. Marine lipids in a cold coastal ecosystem: Gilbert Bay, Labrador. Mar. Biol. 143, 1213-1227.

Copeman, L.A., Stoner, A.W., Ottmar, M.L., Daly, B., Parrish, C.C., Eckert, G.L., 2012. Total lipids, lipid classes, and fatty acids of newly settled red king crab (*Paralithodes camtschaticus*): comparison of hatchery-cultured and wild crabs. J. Shellfish Res. 31, 153-165.

Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus vannamei*. Aquaculture. 147, 261-273.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture. 155, 149-164.

Cutts, C.J., Sawanboonchun, J., de Quero, C.M., Bell, J.G., 2006. Diet-induced differences in the essential fatty acid (EFA) compositions of larval Atlantic cod (Gadus morhua L.) with reference to possible effects of dietary EFAs on larval performance. ICES J. Mar. Sci. 63, 302-310.

Daly, B., Swingle, J.S., Eckert, G.L., 2009. Effects of diet, stocking density, and substrate on survival and growth of hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles in Alaska, USA. Aquaculture. 293, 68-73.

Daly, B., Swingle, J.S., Eckert, G.L., 2012. Increasing hatchery production of juvenile red king crabs (*Paralithodes camtschaticus*) through size grading. Aquaculture. 364, 206-211.

Daly, B., Swingle, J.S., Eckert, G.L., 2013. Dietary astaxanthin supplementation for hatchery-cultured red king crab, *Paralithodes camtschaticus*, juveniles. Aquacult. Nutr. 19, 312-320.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.M., Jeffrey, S.W., 1994. Essential polyunsaturated fatty-acids from 14 species of diatom (*Bacillariophyceae*). Phytochemistry. 35, 155-161.

Epelbaum, A.B., Kovatcheva, N.P., 2005. Daily food intakes and optimal food concentrations for red king crab (*Paralithodes camtschaticus*) larvae fed *Artemia* nauplii under laboratory conditions. Aquacult. Nutr. 11, 455-461.

Epelbaum, A.B., Borisov, R.R., Kovatcheva, N.P., 2006. Early development of the red king crab (*Paralithodes camtschaticus)* from the Barents Sea reared under laboratory conditions: morphology and behaviour. J. Mar. Biol. Assoc. U.K. 86, 317-333.

Fernandez-Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional-value of the rotifer (*Brachionus-plicatilis*). Aquaculture. 112, 195-206.

Figueiredo, J., van Woesik, R., Lin, J., Narciso, L., 2009. *Artemia* franciscana enrichment model - how to keep them small, rich and alive? Aquaculture. 294, 212-220.

Folch, J., Lees, M., Sloane, S.G., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 22, 497-509.

Fraser, A.J., 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Can. J. Fish. Aquat. Sci. 46, 1868-1873.

Gallager, S.M., Mann, R., 1986a. Growth and survival of larvae of *Mercenaria mercenaria* (L) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid-content of eggs. Aquaculture. 56, 105-121.

Gallager, S.M., Mann, R., 1986b. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. Aquaculture. 56, 105-121.

Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in three species of bivalve larvae. Aquaculture. 56, 81-103.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquacult. 1, 71-124.

Goad, L.J., 1981. Sterol biosynthesis and metabolism in marine invertebrates. Pure App. Chem. 53, 837-852.

Hakanson, J.L., 1984. The long and short-term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid-content. Limnol. Oceanogr. 29, 794-804.

Hamasaki, K., Kitada, S., 2008. Potential of stock enhancement for decapod crustaceans. Rev. Fish. Sci. 16, 164-174.

Harding, G.C., Fraser, A.J., 1999. Application of the triacylglycerol/sterol condition index to the interpretation of larval lobster *Homarus americanus* distribution in close proximity to Georges Bank, Gulf of Maine. Mar. Ecol. Prog. Ser. 186, 239-254.

Hassett, R.P., Crockett, E.L., 2009. Habitat temperature is an important determinant of cholesterol contents in copepods. J. Exp. Biol. 212, 71-77.

Lavens, P., Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture. Food and Agriculture Organization (FAO).

Limbourn, A.J., Nichols, P.D., 2009. Lipid, fatty acid and protein content of late larval to early juvenile stages of the western rock lobster, *Panulirus cygnus*. Comp. Biochem. Physiol., B. 152, 292-298.

Lu, Y., Ludsin, S.A., Fanslow, D.L., Pothoven, S.A., 2008. Comparison of three microquantity techniques for measuring total lipids in fish. Can. J. Fish. Aquat. Sci. 65, 2233-2241.

Martins, T.G., Cavalli, R.O., Martino, R.C., Rezende, C.E.M., Wasielesky, W., 2006. Larviculture output and stress tolerance of *Farfantepenaeus paulensis* postlarvae fed *Artemia* containing different fatty acids. Aquaculture. 252, 525-533.

McEvoy, L.A., Navarro, J.C., Hontoria, F., Amat, F., Sargent, J.R., 1996. Two novel *Artemia* enrichment diets containing polar lipid. Aquaculture. 144, 339-352.

Meglen, R.R., 1992. Examining large databases - a chemometric approach using principal component analysis. Mar. Chem. 39, 217-237.

Nates, S.F., McKenney, C.L., 2000. Growth and variations in lipid class and fatty acid composition during larval development of the stone crab, *Menippe adina* Williams and Felder, 1986. Invertebr. Reprod. Dev. 37, 157-165.

Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V., Amat, F., 1999. Lipid conversions during enrichment of *Artemia*. Aquaculture. 174, 155-166.

Nghia, T.T., Wille, M., Vandendriessche, S., TheVinh, Q., Sorgeloos, P., 2007. Influence of highly unsaturated fatty acids in live food on larviculture of mud crab *Scylla paramamosain* (Estampador 1949). Aquacult. Res. 38, 1512-1528.

NPFMC, 2013. Stock assessment and fishery evaluation report for the king and tanner crab fisheries of the Bering sea and Aleutian islands regions. North Pacific Fishery Management Council, Anchorage, AK.

Orensanz, J.M.L., Armstrong, J., Armstrong, D., Hilborn, R., 1998. Crustacean resources are vulnerable to serial depletion: the multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. Rev. Fish Biol. Fish. 8, 117-176.

Ouellet, P., Taggart, C.T., Frank, K.T., 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. Can. J. Fish. Aquat. Sci. 49, 368-378.

Palacios, E., Bonilla, A., Pérez, A., Racotta, I.S., Civera, R., 2004. Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) postlarvae to low salinity. J. Exp. Mar. Biol. Ecol. 299, 201-215.

Parrish, C.C., 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44, 722-731.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, Dordrecht, pp. 309-326.

Parrish, C.C., 2013. Lipids in marine ecosystems. ISRN Oceanography. 2013, 16.

Persselin, S., Daly, B., 2010. Diet and water source effects on larval red king crab cultivation. In: Kruse, G.H., Eckert, G.L., Foy, R., Lipcius, R.N., Sainte-Marie, B., Stram, D.L., Woodby, D. (Eds.), Biology and Management of Exploited Crab Populations Under Climate Change. Alaska Sea Grant, University of Alaska Fairbanks.

Rodriguez-Jaramillo, C., Hurtado, M.A., Romero-Vivas, E., Ramirez, J.L., Manzano, M., Palacios, E., 2008. Gonadal development and histochemistry of the tropical oyster, *Crassostrea corteziensis* (Hertlein, 1951) during an annual reproductive cycle. J. Shellfish Res. 27, 1129-1141.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture. 177, 191-199.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999b. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture. 179, 217-229.

Sasaki, G.C., 1984. Biochemical changes associated with embryonic and larval development in the American lobster *Homarus americanus* Milne Edwards. Massachusetts Institute of Technology and Woods Hole Oceanographic Institution.

Sasaki, G.C., Capuzzo, J.M., Biesiot, P., 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. Can. J. Fish. Aquat. Sci. 43, 2311-2319.

Sheen, S., Wu, S., 2002. Essential fatty acid requirements of juvenile mud crab, *Scylla serrata* (Forskål, 1775)(Decapoda, scyllaridae). Crustaceana. 75, 1387-1401.

Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture. 200, 147-159.

Stevens, B.G., 2012. Feeding rate of juvenile red king crabs, *Paralithodes camtschaticus*, in the laboratory: effects of temperature, size, molting, and feeding frequency. Polar Biol. 35, 1791-1799.

Stevens, B.G., Kittaka, J., 1998. Postlarval settling behavior, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus*. Mar. Ecol. Prog. Ser. 167, 197-206.

Stevens, B.G., Persselin, S., Matweyou, J., 2008. Survival of blue king crab *Paralithodes platypus* Brandt, 1850, larvae in cultivation: effects of diet, temperature and rearing density. Aquacult. Res. 39, 390-397.

Stevens, C.J., Deibel, D., Parrish, C.C., 2004. Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). Mar. Biol. 144, 905-915.

Stoner, A.W., Ottmar, M.L., Haines, S.A., 2010. Temperature and habitat complexity mediate cannibalism in red king crab: observation on activity, feeding, and prey defense mechanisms. J. Shellfish Res. 29, 1005-1012.

Sui, L., Wille, M., Cheng, Y., Sorgeloos, P., 2007. The effect of dietary n-3 HUFA levels and DHA/EPA ratios on growth, survival and osmotic stress tolerance of Chinese mitten crab *Eriocheir sinensis* larvae. Aquaculture. 273, 139-150.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K., 2004a. Essential fatty acids for larval mud crab *Scylla serrata*: implications of lack of the ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. Aquaculture. 231, 403-416.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K., 2004b. Effects of *Artemia* enriched with eicosapentaenoic and docosahexaenoic acid on survival and occurrence of molting failure in megalop larvae of the mud crab *Scylla serrata*. Fish. Sci. 70, 650-658.

Swingle, J.S., Daly, B., Hetrick, J., 2013. Temperature effects on larval survival, larval period, and health of hatchery-reared red king crab, *Paralithodes camtschaticus*. Aquaculture. 384, 13-18.

Takeuchi, T., Nakamoto, Y., Hamasaki, K., Sekiya, S., Watanabe, T., 1999. Requirement of n-3 highly unsaturated fatty acids for larval swimming crab *Portunus trituberculatus*. Nippon Suisan Gakkaishi. 65, 797-803.

Tziouveli, V., Smith, G.G., 2012. A comparison of the fatty acid profiles of adult tissues, and newly hatched, fed and starved *Lysmata amboinensis* larvae. Aquacult. Res. 43, 577-587.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture. 124, 223-251.

Wen, X.B., Chen, L.Q., Ku, Y.M., Zhou, K.Y., 2006. Effect of feeding and lack of food on the growth, gross biochemical and fatty acid composition of juvenile crab, *Eriocheir sinensis*. Aquaculture. 252, 598-607.

Xu, X.L., Ji, W.J., Castell, J.D., Odor, R.K., 1994. Essential fatty-acid requirement of the chinese prawn, *Penaeus-chinensis*. Aquaculture. 127, 29-40.

Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V., Zohar, Y., 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. Aquaculture. 244, 129-139.

Zohar, Y., Hines, A.H., Zmora, O., Johnson, E.G., Lipcius, R.N., Seitz, R.D., Eggleston, D.B., Place, A.R., Schott, E.J., Stubblefield, J.D., Chung, J.S., 2008. The Chesapeake Bay blue crab (*Callinectes sapidus*): A multidisciplinary approach to responsible stock replenishment. Rev. Fish. Sci. 16, 24-34.

General Conclusions

Over the last 40 years significant research effort has been focused on determining the lipid nutritional requirements of larval stages for commercially important fish and shellfish (Bell et al., 1986; Castell et al., 2003; Copeman et al., 2014; Copeman and Laurel, 2010; Gimenez and Anger, 2005; New, 1976; Suprayudi et al., 2004; Theriault and Pernet, 2007). I reviewed the importance of lipids and EFAs for hatchery rearing in Chapter 1. Fatty acid nutrition plays a vital role in the survival, growth, and stress tolerance of marine organisms (Andres et al., 2010; Coutteau et al., 1996). Often natural prey items are not easily raised in captivity (Figueiredo et al., 2009), leading to the use of organisms that are easy to culture in large amounts. However, these organisms must be enriched to provide the proper nutrition for the intended predator (Fernandez-Reiriz et al., 1993; Figueiredo et al., 2009; Sorgeloos et al., 2001). Long-chain PUFA are important in molting success, growth, ability to handle stress, and overall survival of crustaceans in a hatchery (Andres et al., 2010; Coutteau et al., 1996; Holme et al., 2009). Specifically, dietary FAs DHA, EPA, and AA have been linked to higher hatchery success for a number of crustaceans, including red king crab (Holme et al., 2009; Kittaka et al., 2002; Mercier et al., 2009). Although the ideal ratios of these FAs vary depending on the species, they have all proven important in hatchery success.

Experiments in Chapter 2 explored the importance of these FAs for king crab larvae using enrichments based on ratios found in Copeman et al. (2012), where FA composition of wild and hatchery-raised juveniles was explored. Although survival did not vary among the diets, differences were found during this study. Biochemical composition of EFAs was significantly different in crabs from all four dietary treatments during the fourth zoeal, glaucothoe, and first juvenile stages. Crabs that received EFAs in their diets recovered faster from a salinity stressor

and crabs receiving DHA & EPA and DHA & AA diets were found to have more and larger lipid droplets than larvae that received either the DHA or control (OA) diets. Slower recovery from a fresh water stress test suggests that EFAs may improve performance during stress.

Aquaculture could potentially be useful in the RKC stock enhancement effort. King crab larvae have been successfully raised to the juvenile stage in the past, and this current study contributes information on the impacts of EFAs ratios on RKC larvae growth and survival along with tools to assess condition of cultured crab. These results advance our understanding of crustacean larval nutrition and inform the feasibility of providing EFAs to king crab raised in hatcheries. Future studies could examine the biochemistry of wild king crab larval stages, specifically the glaucothoe stage where a bottleneck occurs, to better determine the ratios of FAs needed at each larval stage. Comparisons among stocks may also provide insight on how environmental conditions impact dietary needs. Developing diets specific to king crab could increase hatchery success and lead to better-conditioned organisms to be used to help recover wild stocks. This study is the first step in creating a diet to be used to raise king crab in hatcheries.

References

Andres, M., Estevez, A., Hontoria, F., Rotllant, G., 2010. Differential utilization of biochemical components during larval development of the spider crab *Maja brachydactyla* (Decapoda: Majidae). Mar. Biol. 157, 2329-2340.

Bell, M.V., Henderson, R.J., Sargent, J.R., 1986. The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol., B. 83, 711-719.

Castell, J., Blair, T., Neil, S., Howes, K., Mercer, S., Reid, J., Young-Lai, W., Gullison, B., Dhert, P., Sorgeloos, P., 2003. The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*Brachionus plicatilis*) fed to larval haddock (*Melanogrammus aeglefinus*). Aquacult. Int. 11, 109-117.

Copeman, L., Daly, B., Eckert, G.L., Swingle, J., 2014. Storage and utilization of lipid classes and fatty acids during the early ontogeny of blue king crab, Paralithodes platypus. Aquaculture. 424, 86-94.

Copeman, L.A., Laurel, B.J., 2010. Experimental evidence of fatty acid limited growth and survival in Pacific cod larvae. Mar. Ecol. Prog. Ser. 412, 259-272.

Copeman, L.A., Stoner, A.W., Ottmar, M.L., Daly, B., Parrish, C.C., Eckert, G.L., 2012. Total lipids, lipid classes, and fatty acids of newly settled red king crab (*Paralithodes camtschaticus*): comparison of hatchery-cultured and wild crabs. J. Shellfish Res. 31, 153-165.

Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus vannamei*. Aquaculture. 147, 261-273.

Fernandez-Reiriz, M.J., Labarta, U., Ferreiro, M.J., 1993. Effects of commercial enrichment diets on the nutritional-value of the rotifer (*Brachionus-plicatilis*). Aquaculture. 112, 195-206.

Figueiredo, J., van Woesik, R., Lin, J., Narciso, L., 2009. *Artemia* franciscana enrichment model - how to keep them small, rich and alive? Aquaculture. 294, 212-220.

Gimenez, L., Anger, K., 2005. Effects of temporary food limitation on survival and development of *Brachyuran* crab larvae. J. Plankton Res. 27, 485-494.

Holme, M.H., Brock, I., Southgate, P.C., Zeng, C.S., 2009. Effects of starvation and feeding on lipid class and fatty acid profile of late stage mud crab, *Scylla serrata*, larvae. J. World Aquacult. Soc. 40, 493-504.

Kittaka, J., Stevens, B.G., Teshima, S., Ishikawa, M., 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. In: Paul, A.J., Dawe, E.G., Elner, R., Jamieson, G.S., Kruse, G.H., Otto, R.S., Sainte-Marie, B., Shirley, T.C., Woodby, D. (Eds.), Crabs in Cold Water Regions: Biology, Management, and Economics.

Mercier, L., Racotta, I.S., Yepiz-Plascencia, G., Muhlia-Almazan, A., Civera, R., Quinones-Arreola, M.F., Wille, M., Sorgeloos, P., Palacios, E., 2009. Effect of diets containing different levels of highly unsaturated fatty acids on physiological and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* (Boone) exposed to handling stress. Aquacult. Res. 40, 1849-1863.

New, M.B., 1976. A review of dietary studies with shrimp and prawns. Aquaculture. 9, 101-144.

Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture. 200, 147-159.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K., 2004. Effects of *Artemia* enriched with eicosapentaenoic and docosahexaenoic acid on survival and occurrence of molting failure in megalop larvae of the mud crab *Scylla serrata*. Fish. Sci. 70, 650-658.

Theriault, I., Pernet, F., 2007. Lipid nutrition and settlement behaviour in American lobster *Homarus americanus*. Aquat. Bio. 1, 121-133.